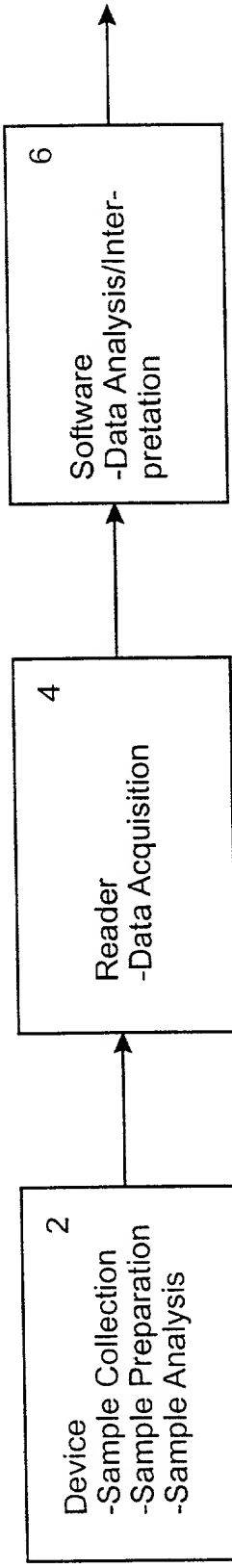

2
Device
-Sample Collection
-Sample Preparation
-Sample Analysis
4
Reader
-Data Acquisition
6
Software
-Data Analysis/Inter-
pretation

FIG. 1

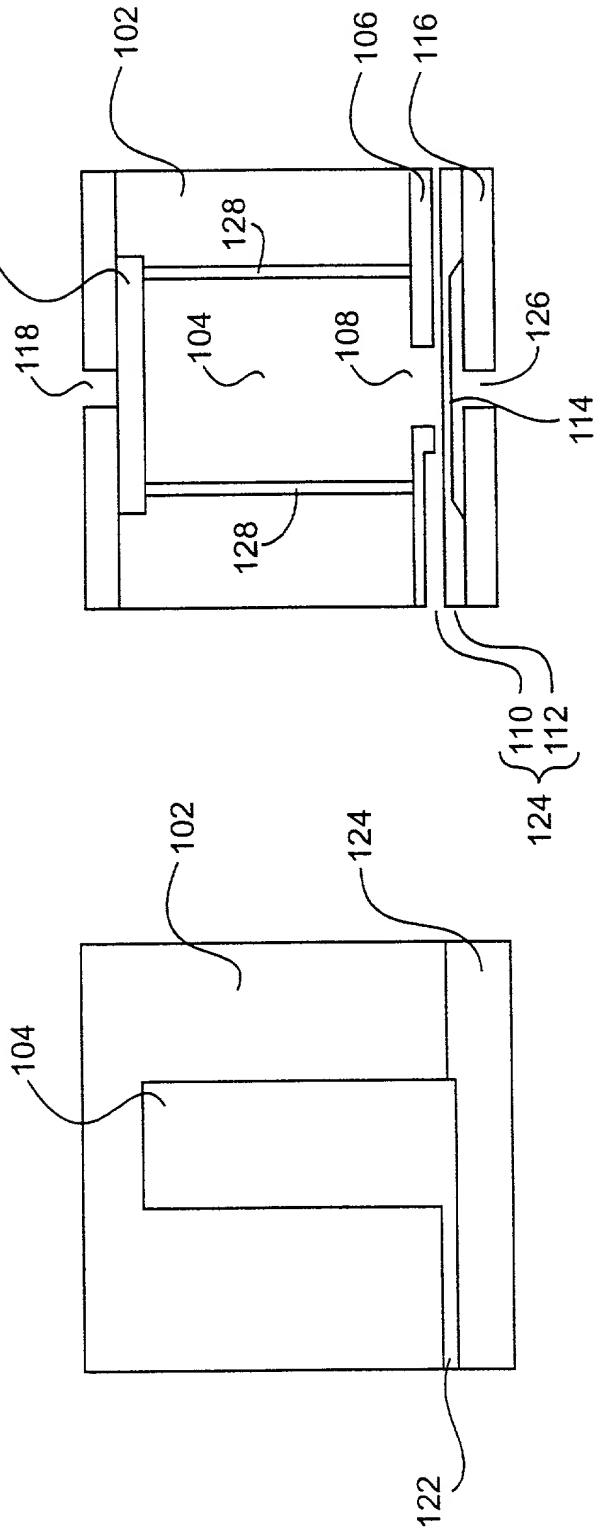


FIG. 2b

FIG. 2a

FIG. 2a & 2b

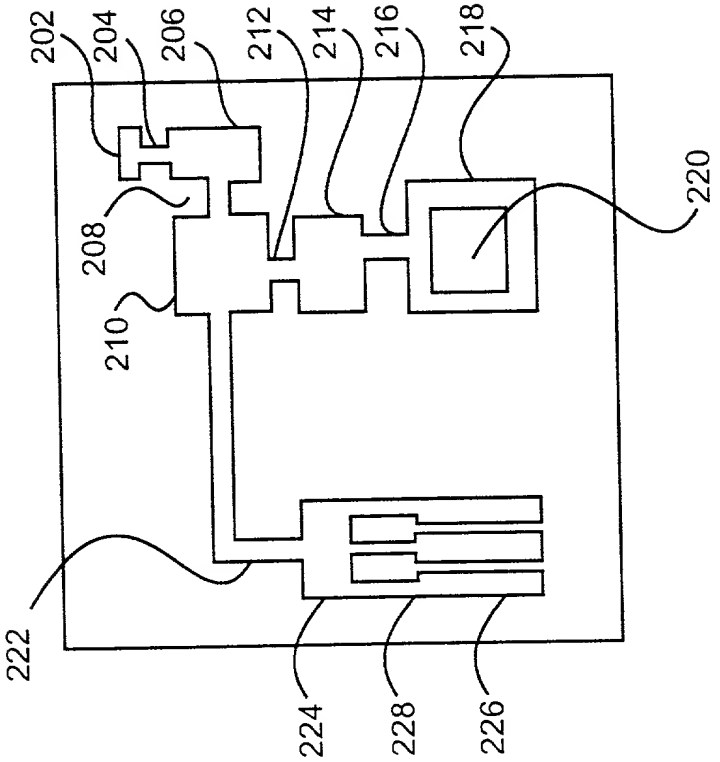


FIG. 3

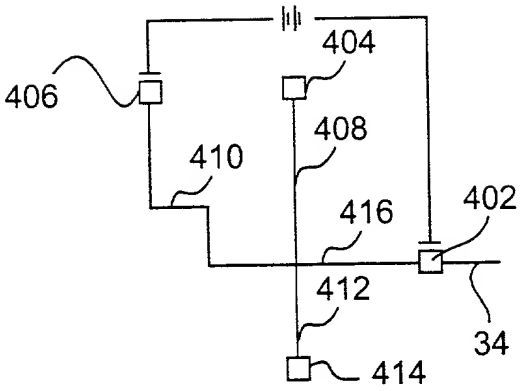


FIG. 4a

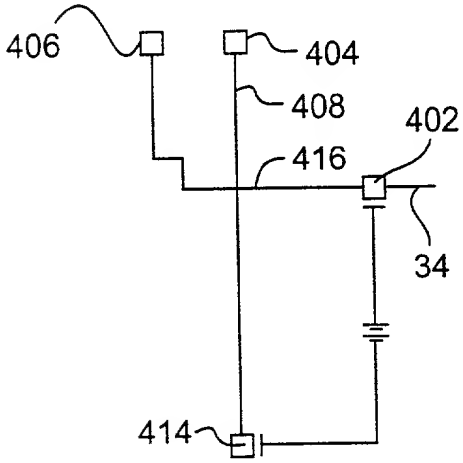


FIG. 4b

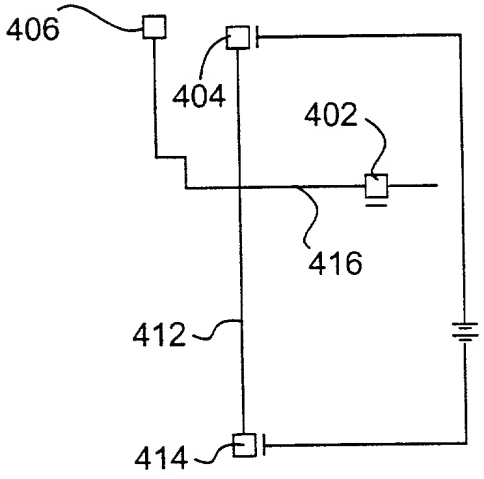


FIG. 4c

FIG. 4a-4c

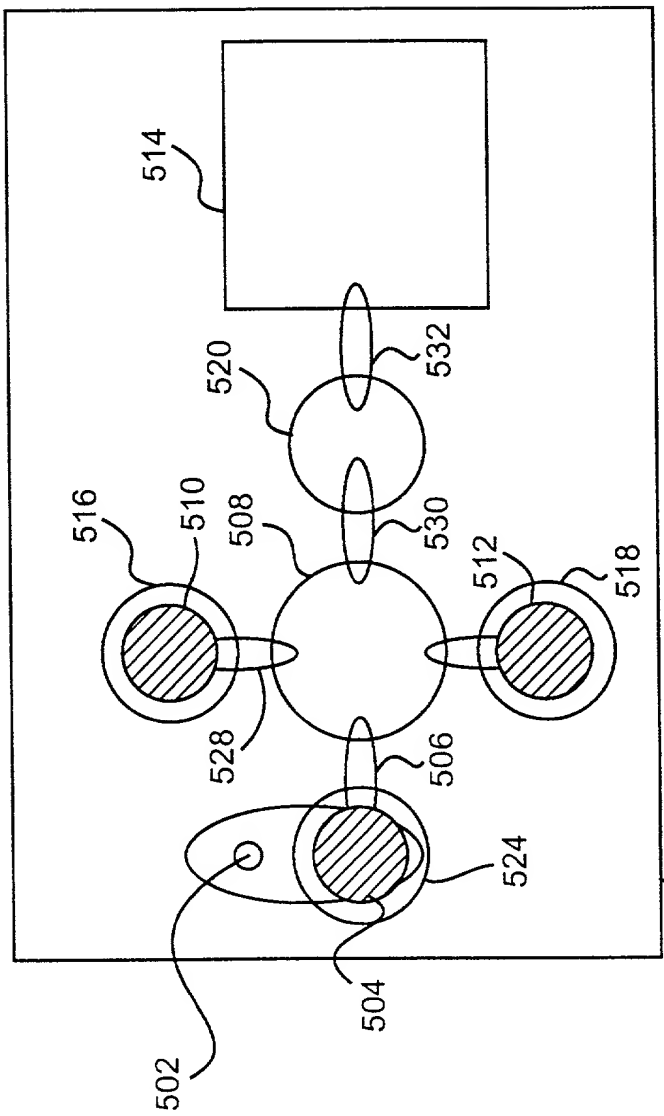


FIG. 5a

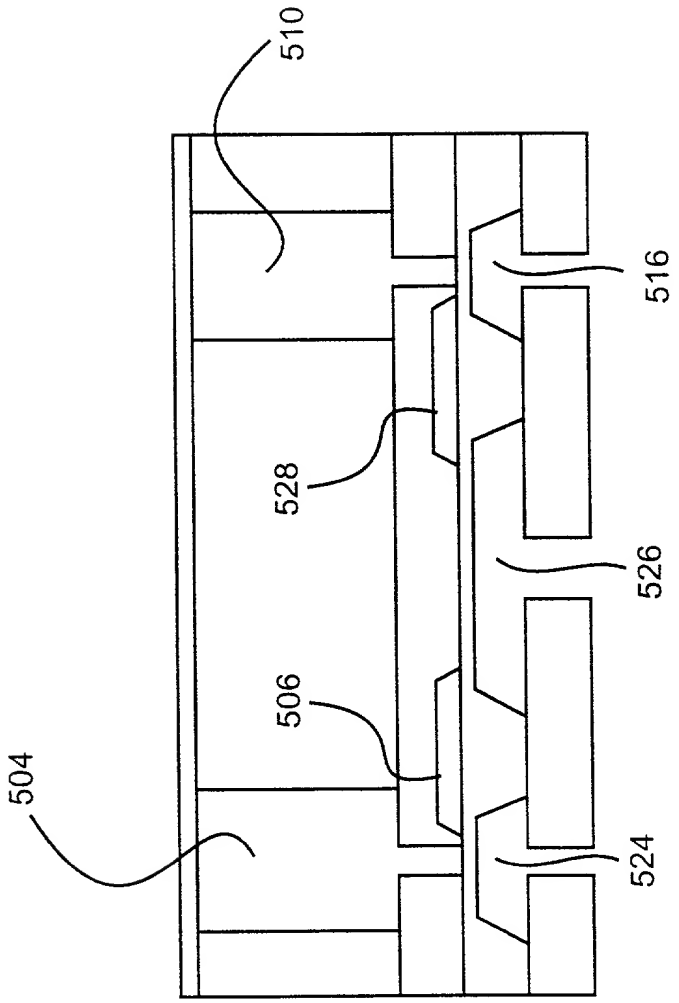


FIG. 5b

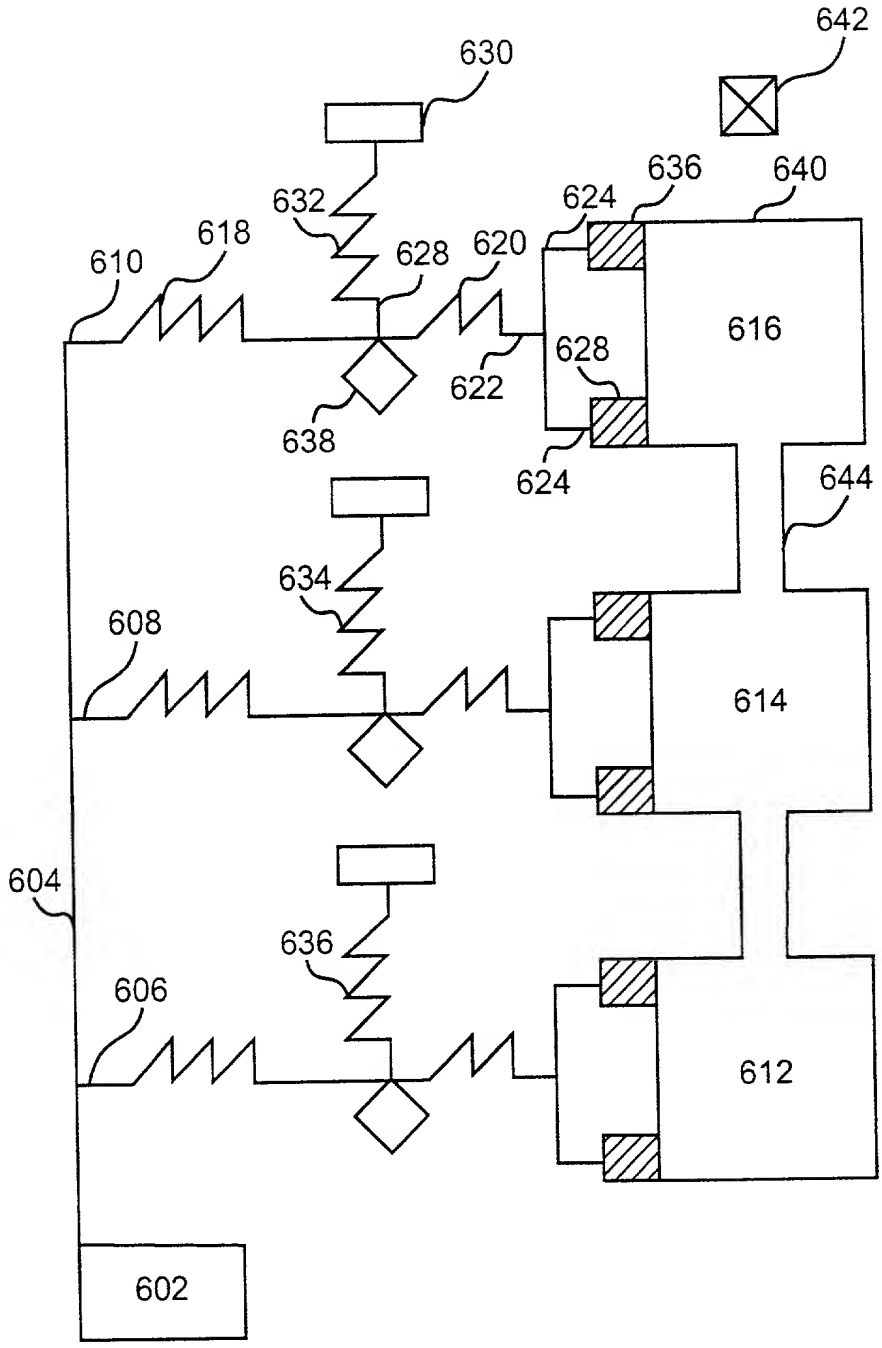

642
630
636
640
632
624
618
610
628
620
616
628
622
638
624
644
634
608
614
604
636
606
612
602

FIG. 6a

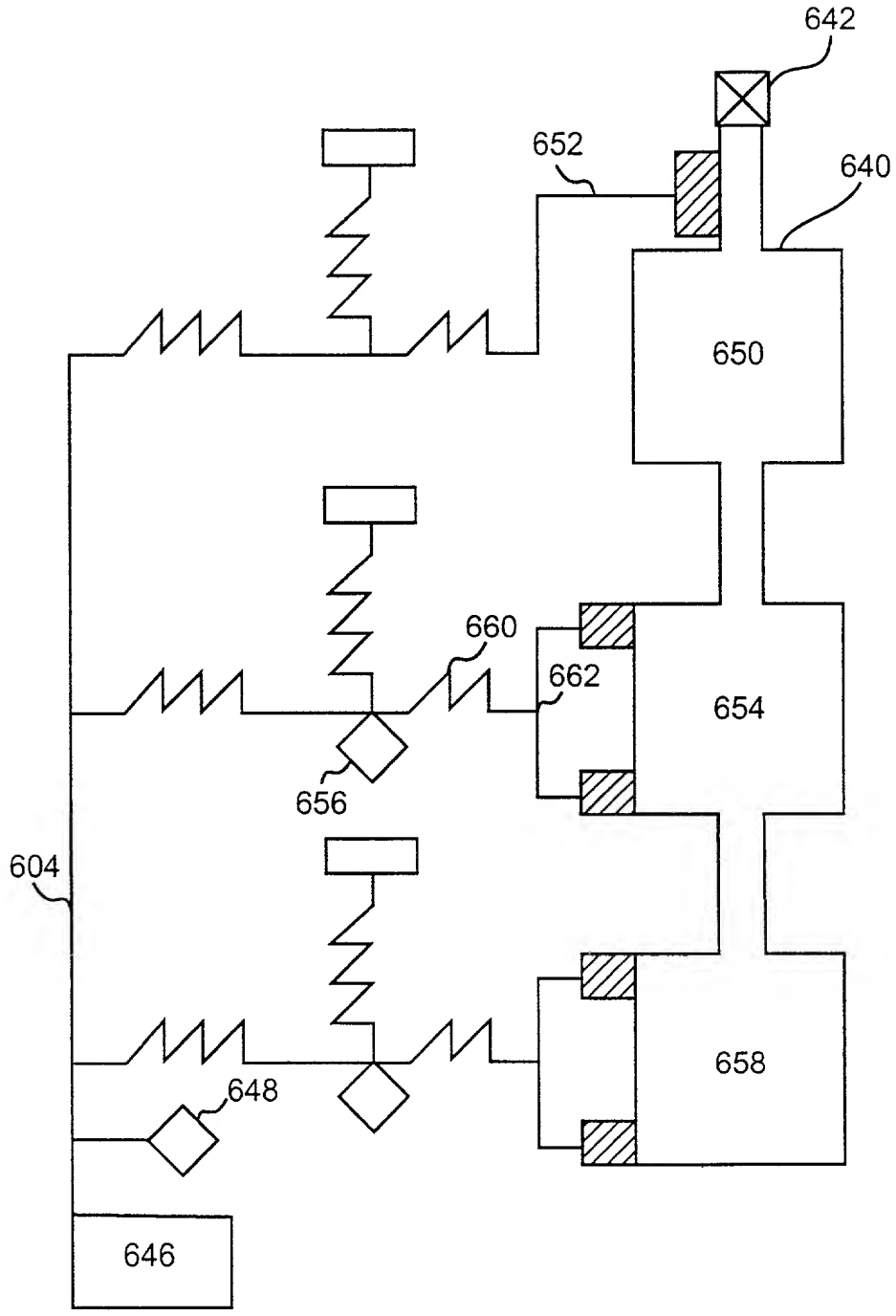


FIG. 6b

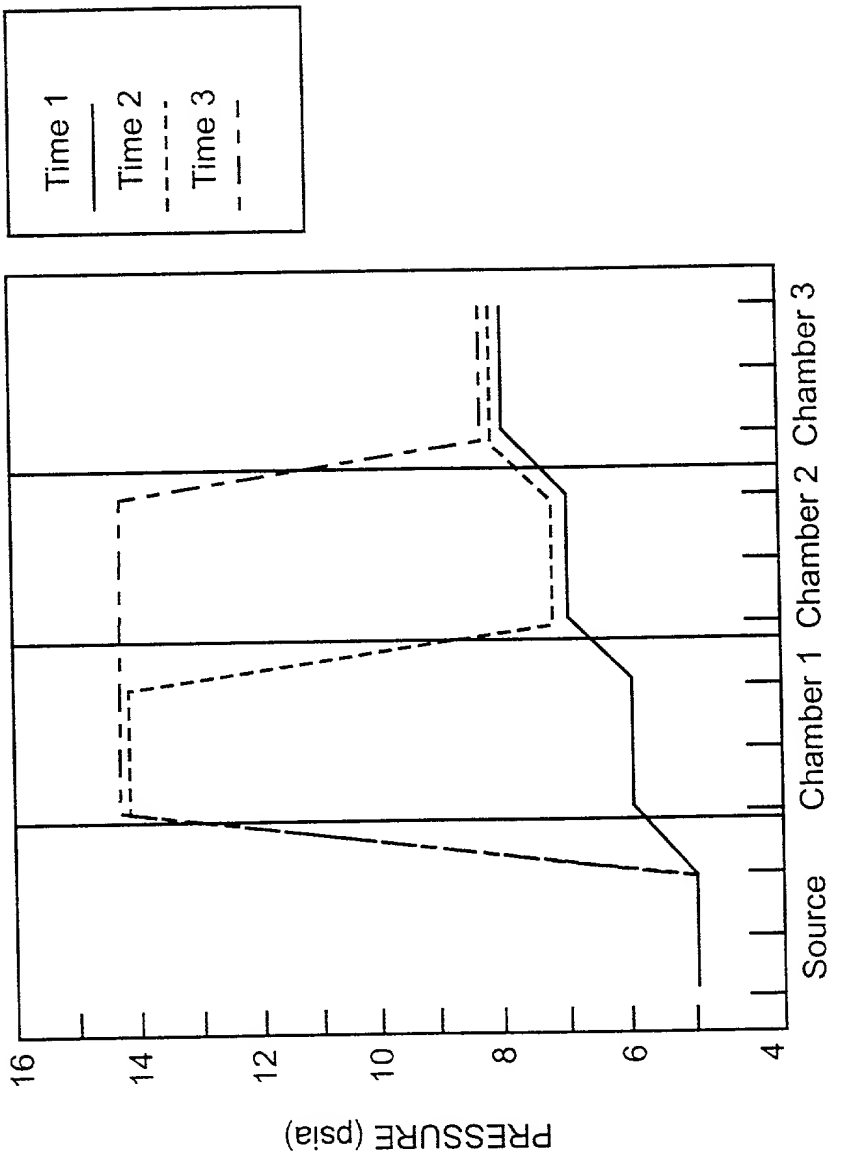

Time 1
Time 2
Time 3
PRESSURE (psia)
16
14
12
10
8
6
4
Source
Chamber 1
Chamber 2
Chamber 3
POSITION

FIG. 6C

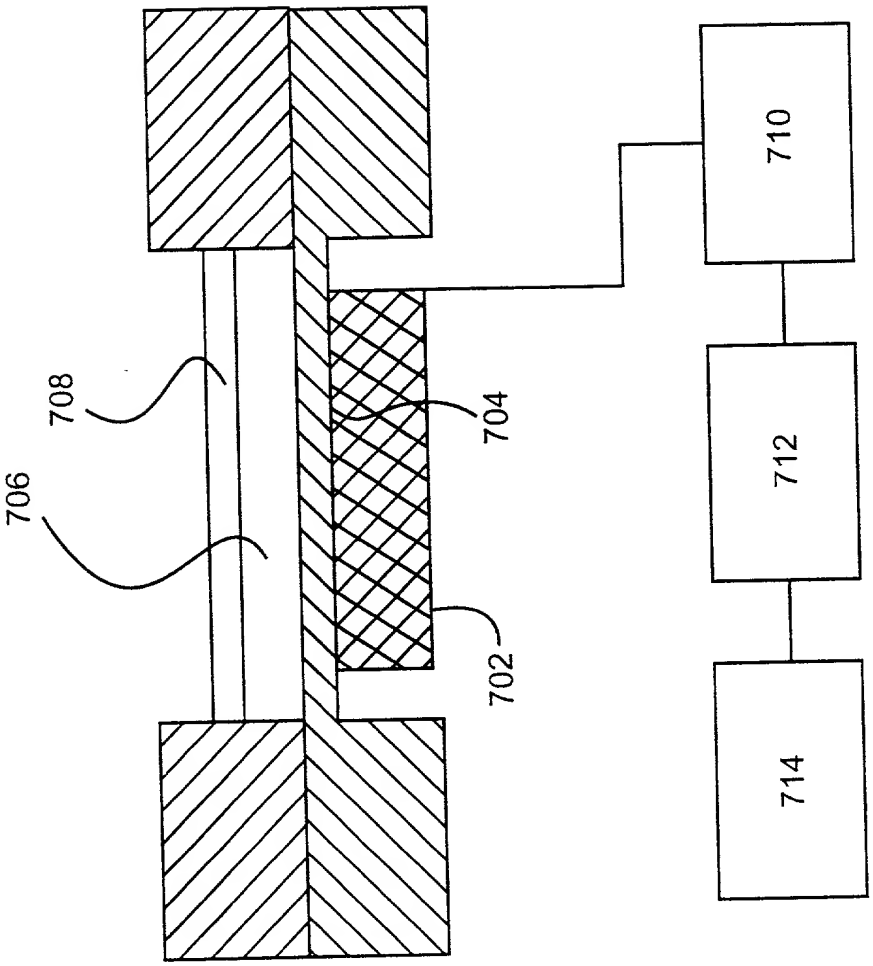


FIG. 7a

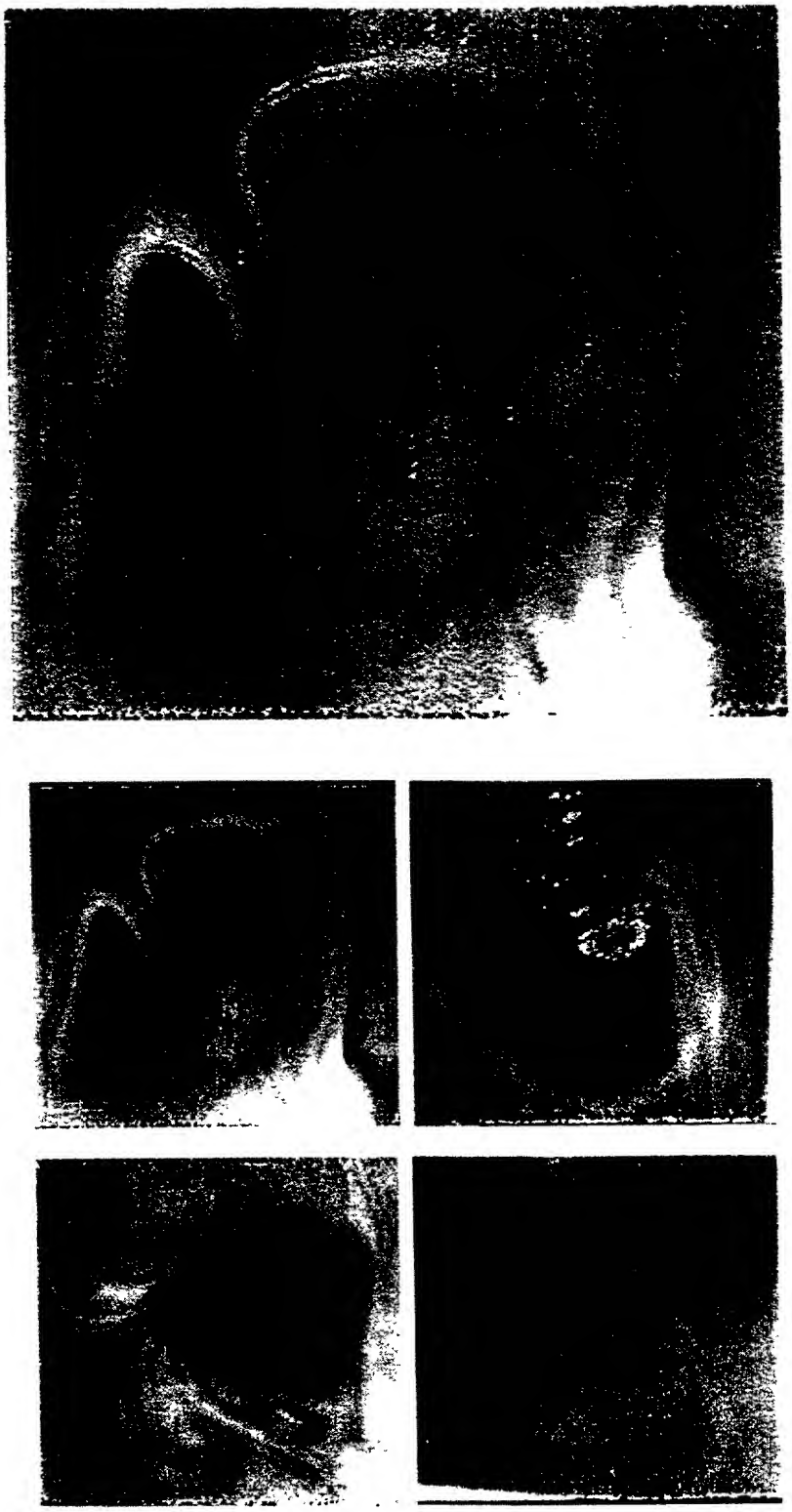


FIG. 7B

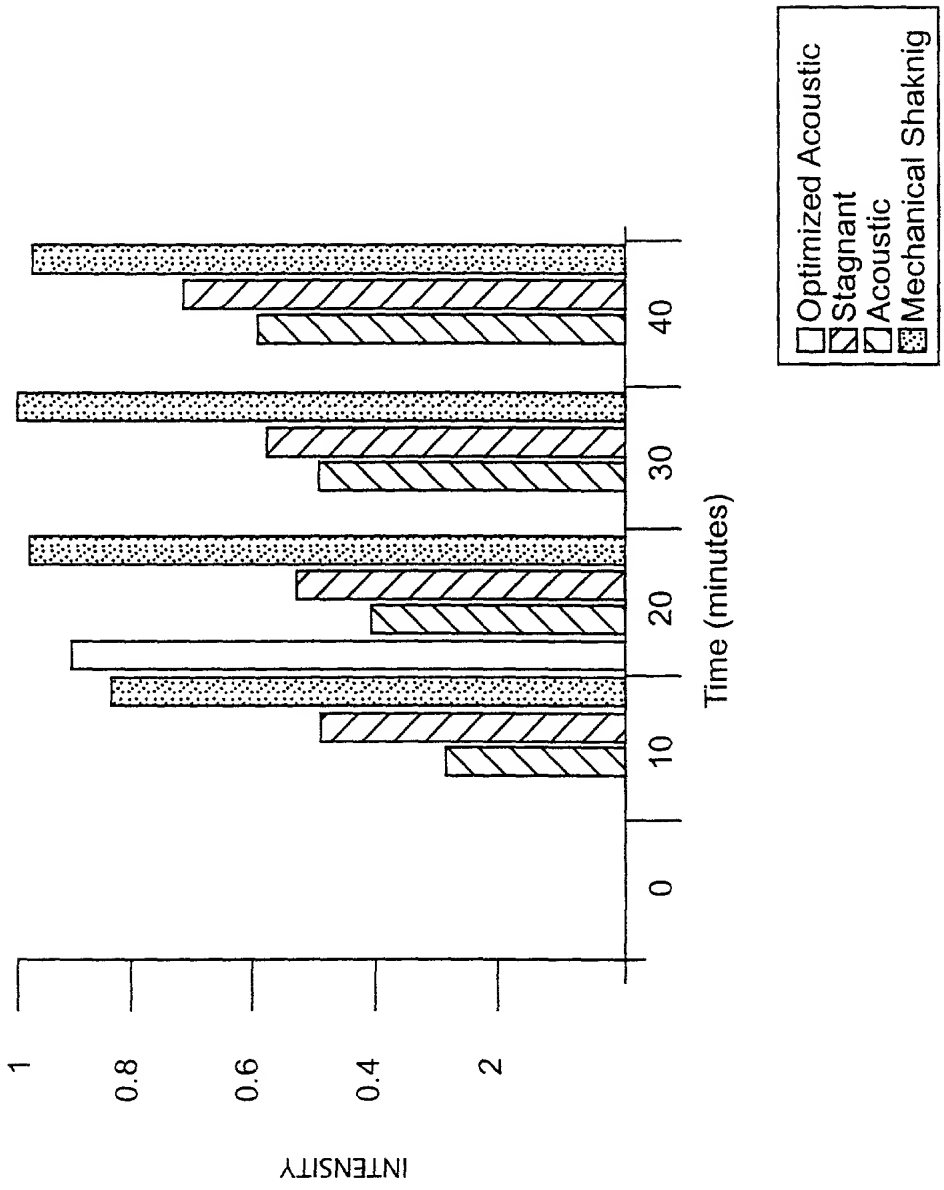

Optimized Acoustic
Stagnant
Acoustic
Mechanical Shaknig
Time (minutes)
0
10
20
30
40
INTENSITY
1
0.8
0.6
0.4
2

FIG. 7c

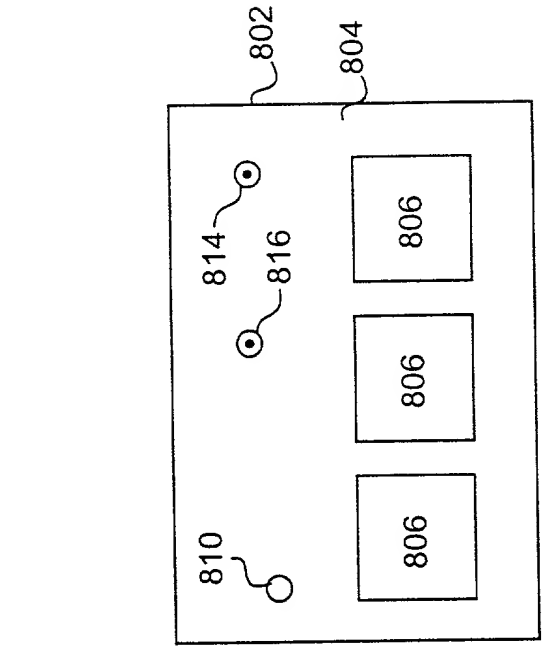


FIG. 8b

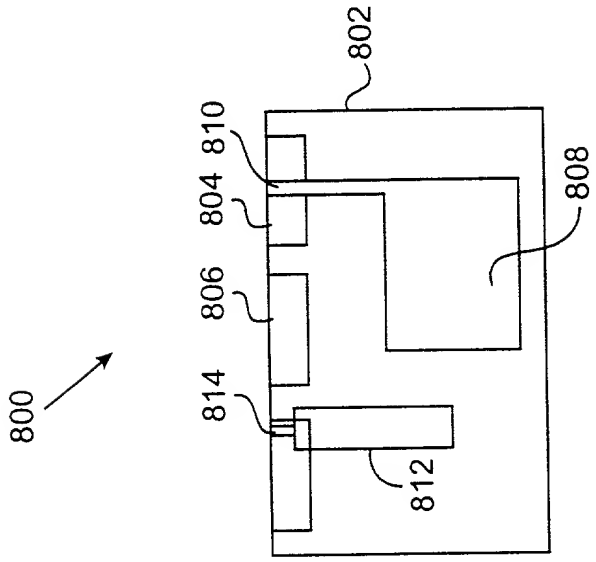
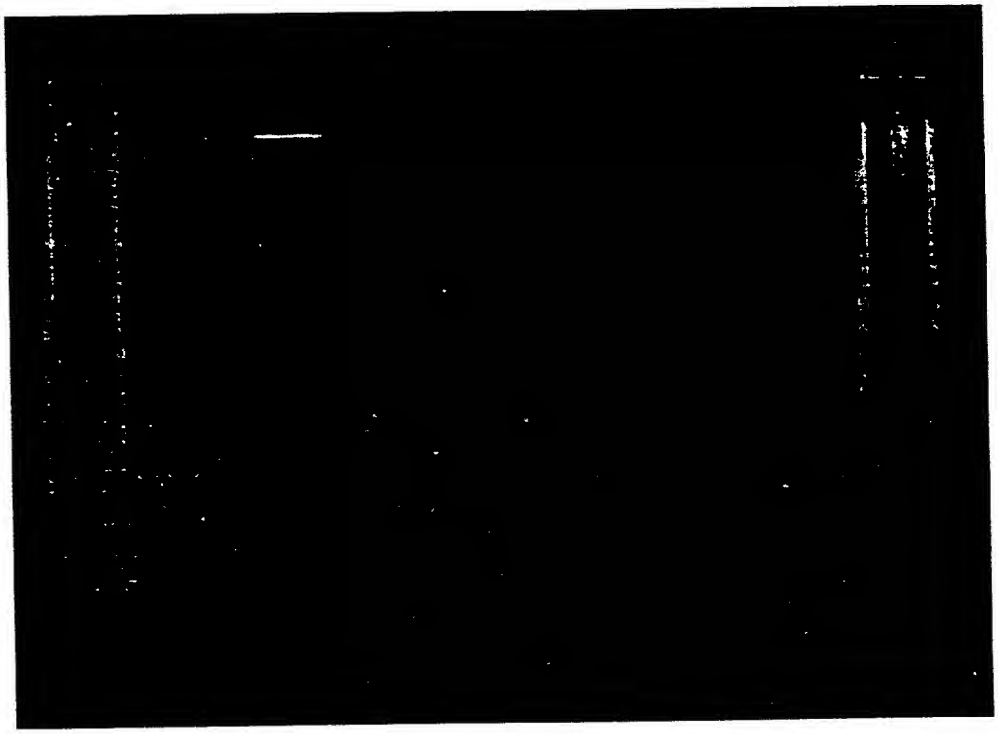


FIG. 8a

FIG. 8a & 8b

t = 0 5 10 30 60 120 minutes



Correct Call Rates:

74% 95.8% 95.9%

95.9% 95.5% 83%

FIG. 9a

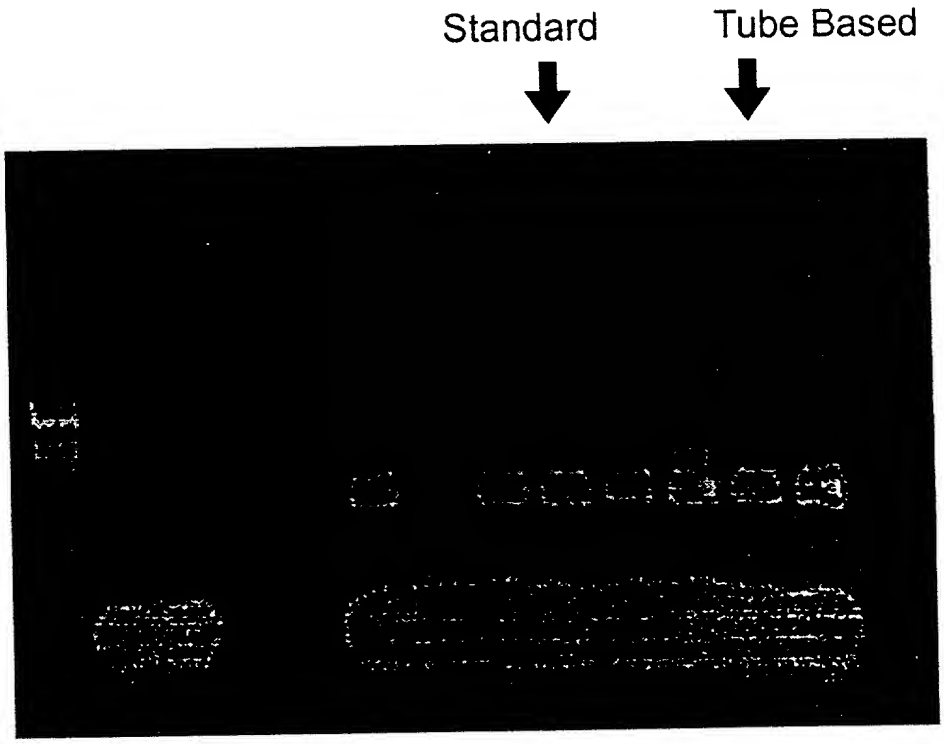


FIG. 9b

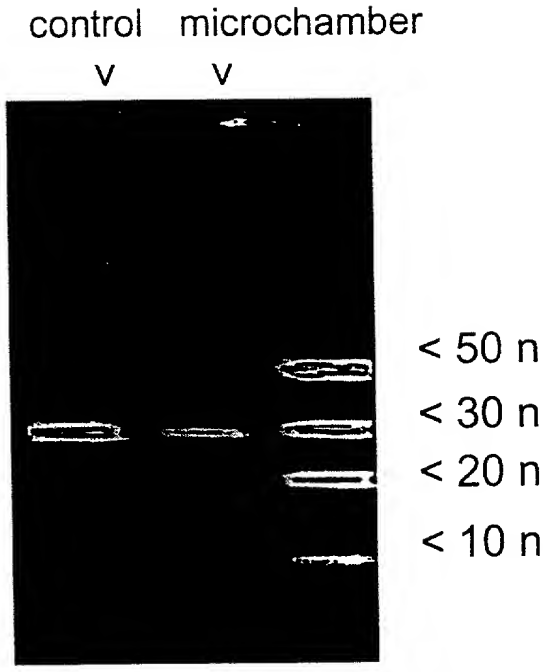


FIG. 9c

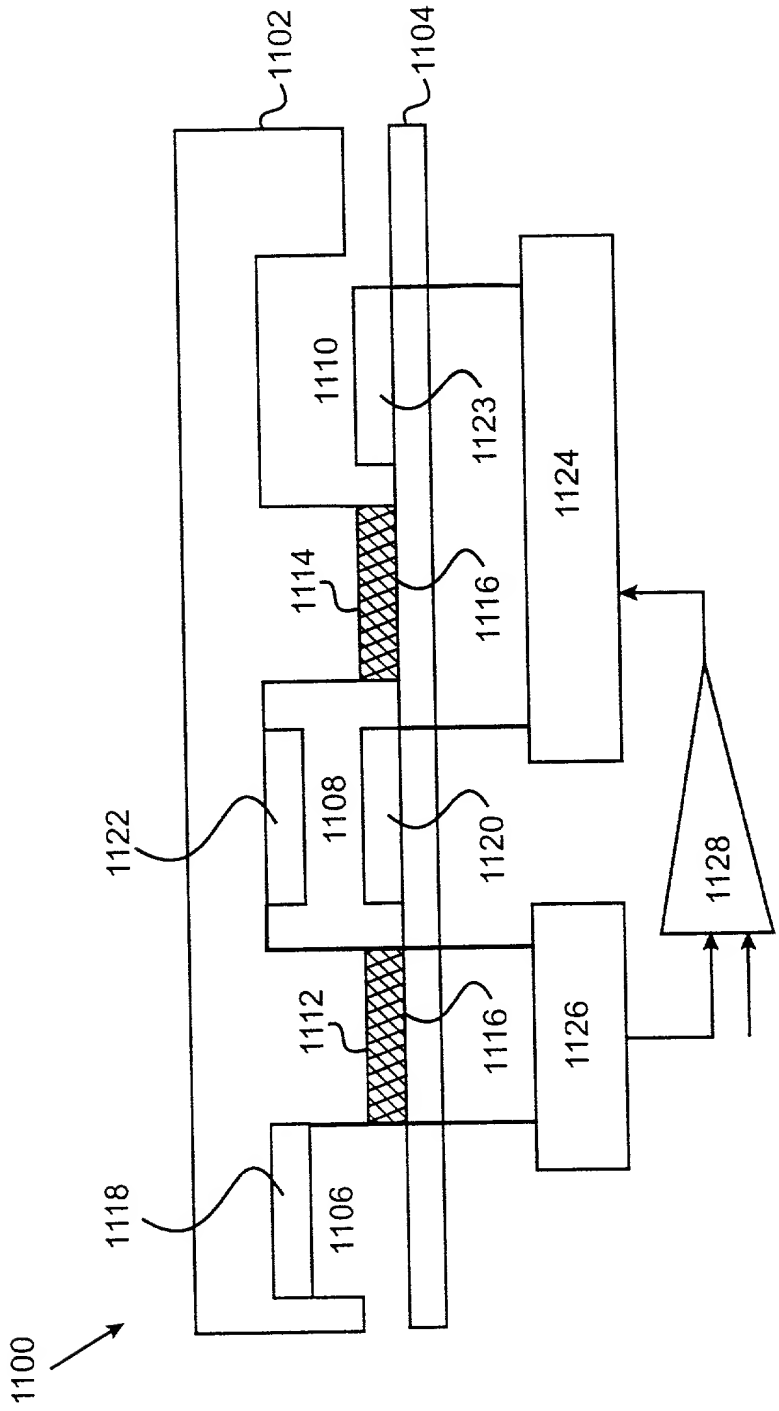

1100
1102
1104
1106
1108
1110
1112
1114
1116
1116
1118
1120
1122
1123
1124
1126
1128

FIG. 10

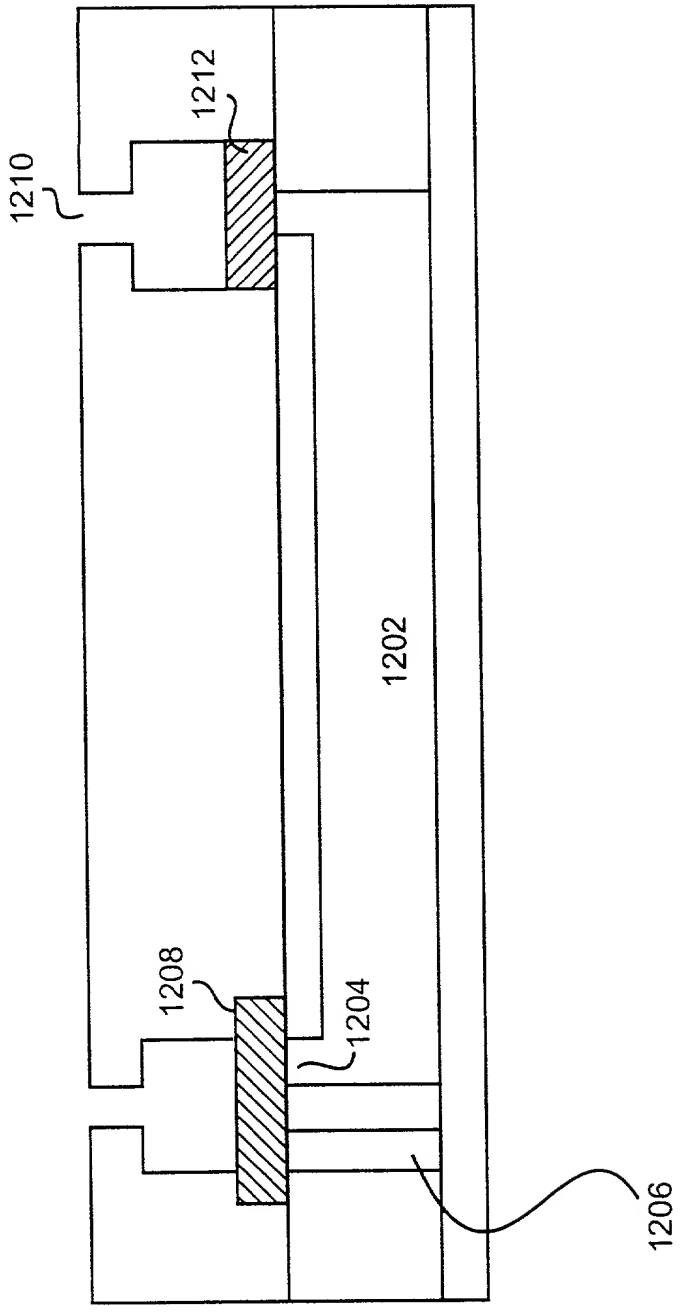


FIG. 11A

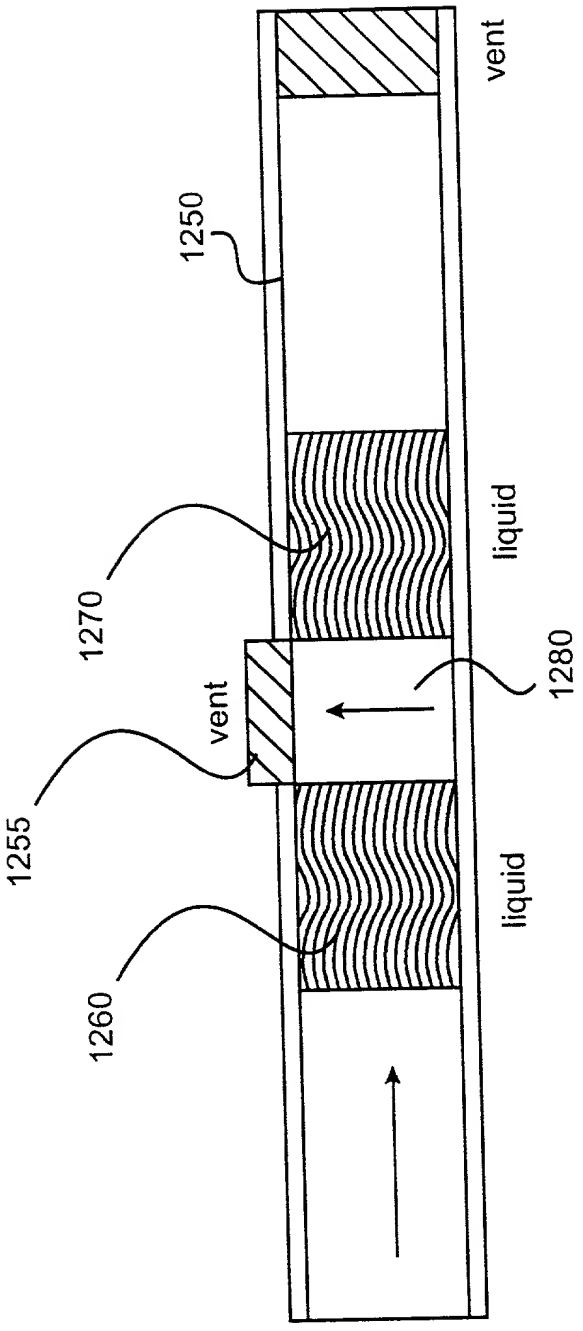


FIG. 11b

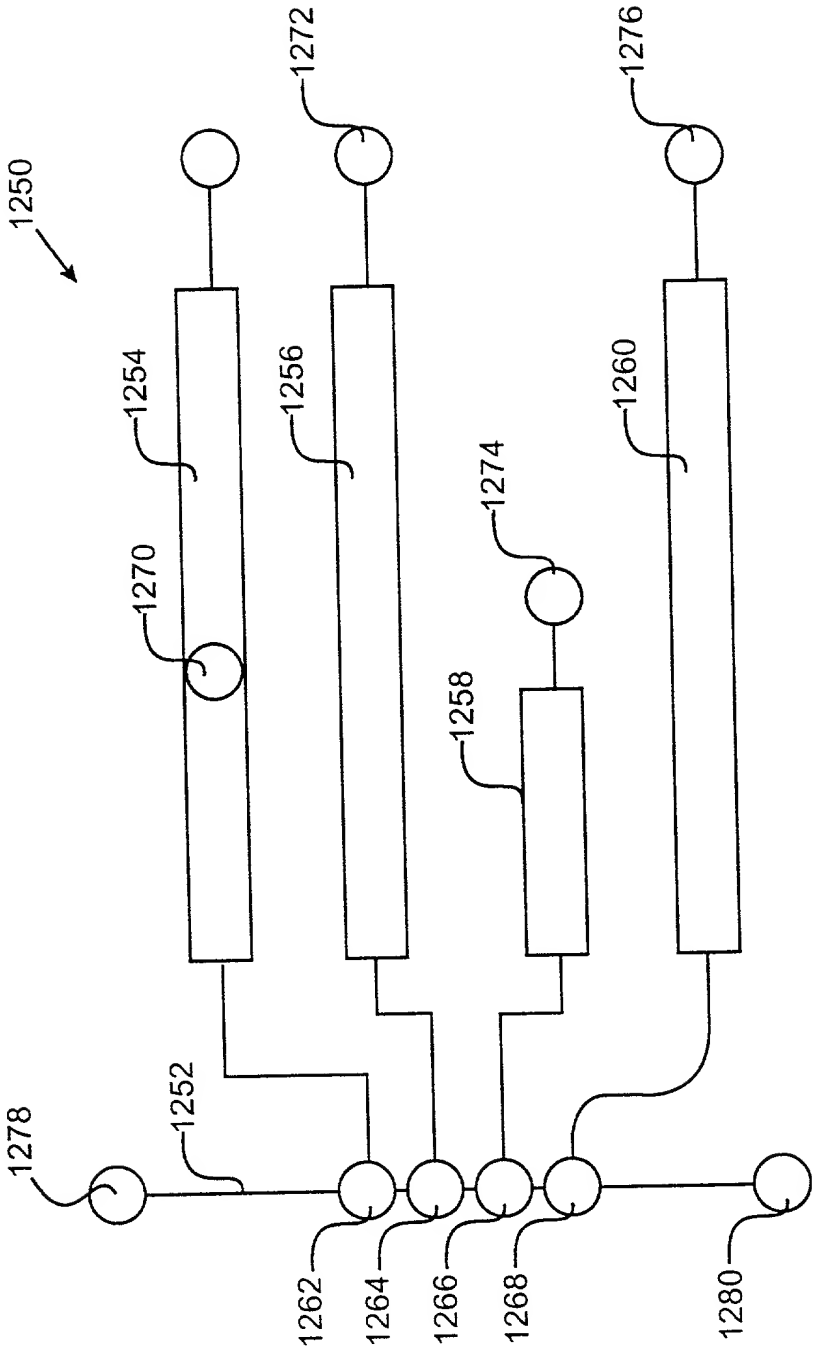

1250
1254
1256
1258
1260
1262
1264
1266
1268
1270
1272
1274
1276
1278
1280
1252

FIG. 11c

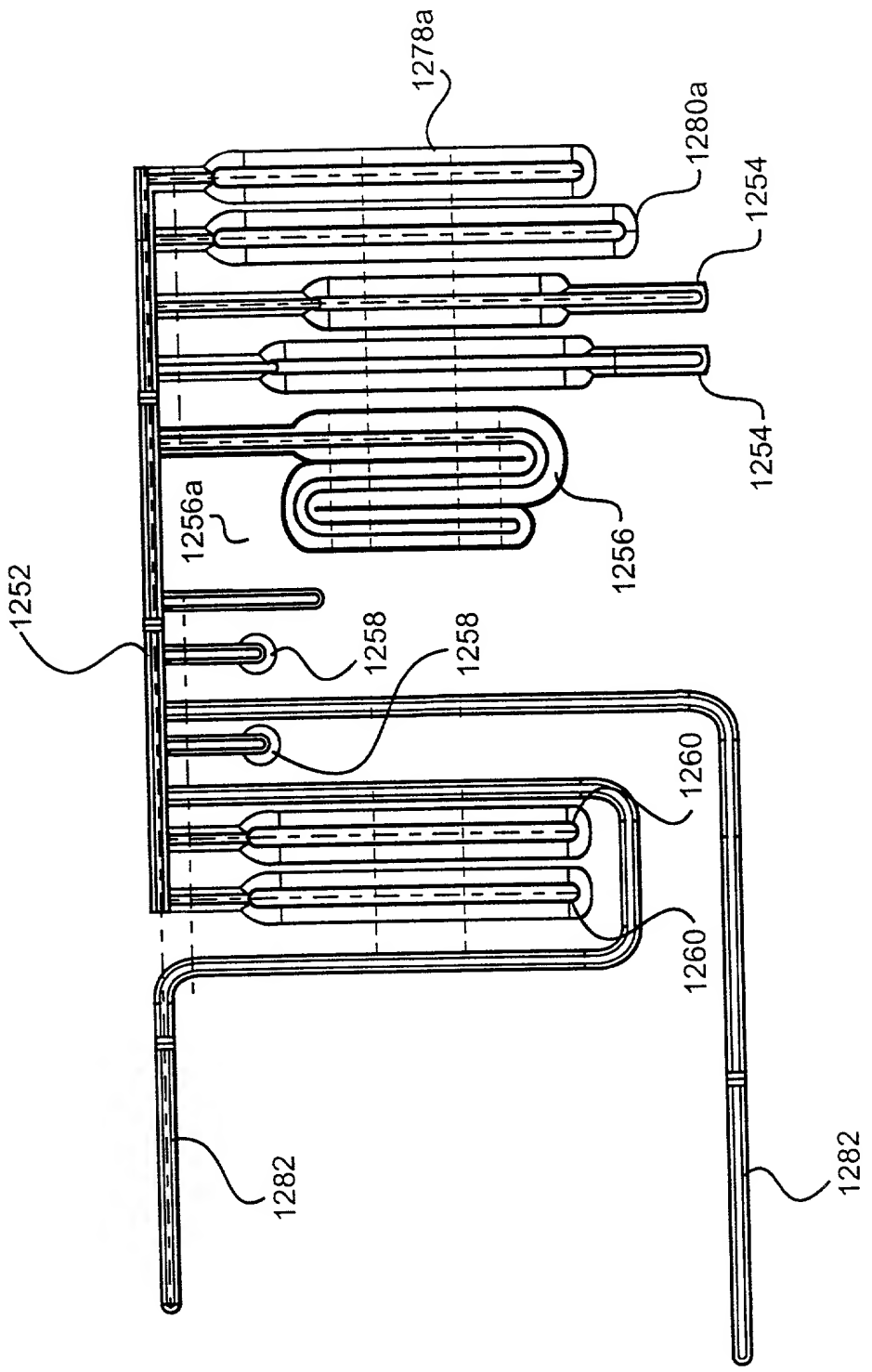

1278a
1280a
1254
1254
1256
1256a
1252
1258
1258
1260
1260
1282
1282

FIG. 11d

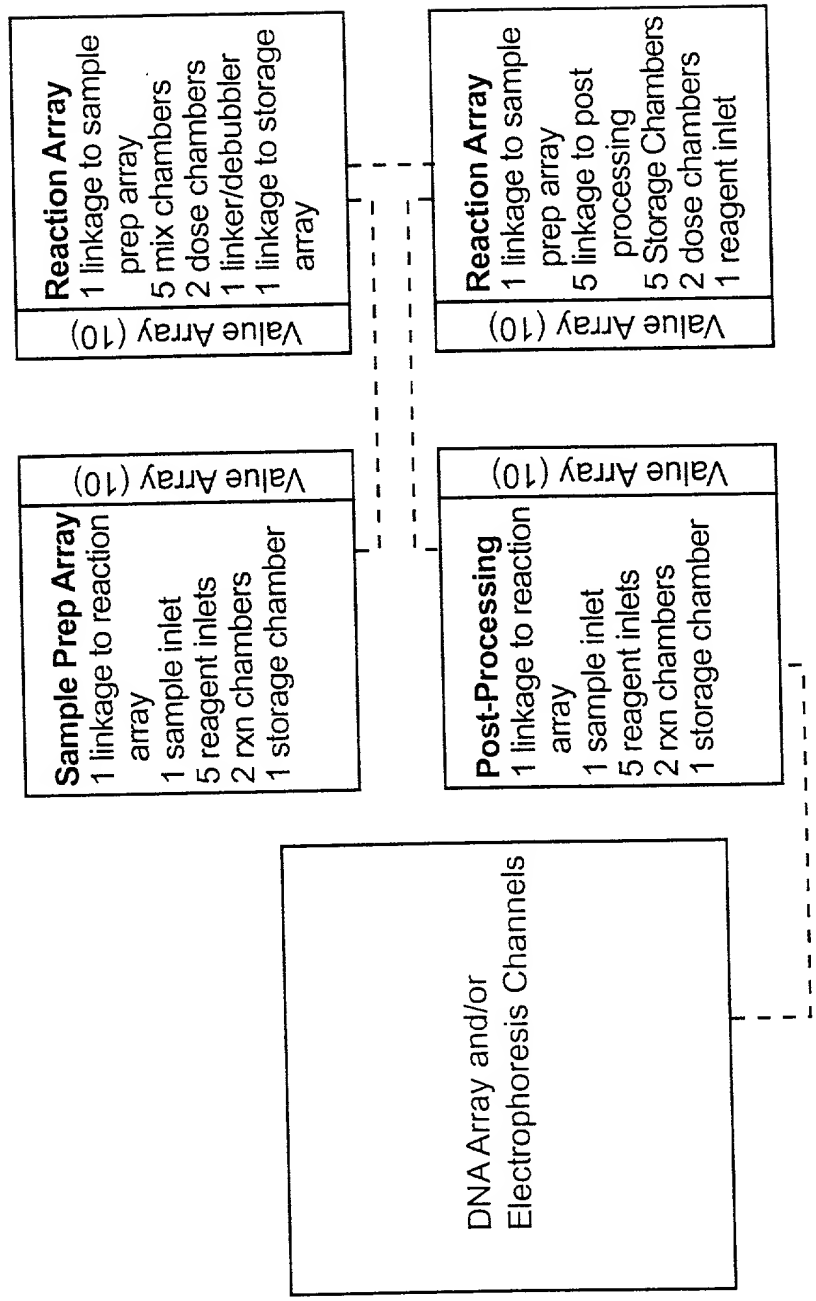

Reaction Array
1 linkage to sample prep array
5 mix chambers
2 dose chambers
1 linker/debubbler
1 linkage to storage array
Value Array (10)
Sample Prep Array
1 linkage to reaction array
1 sample inlet
5 reagent inlets
2 rxn chambers
1 storage chamber
Value Array (10)
Post-Processing
1 linkage to reaction array
1 sample inlet
5 reagent inlets
2 rxn chambers
1 storage chamber
Value Array (10)
Reaction Array
1 linkage to sample prep array
5 linkage to post processing
5 Storage Chambers
2 dose chambers
1 reagent inlet
Value Array (10)
DNA Array and/or
Electrophoresis Channels

FIG. 12

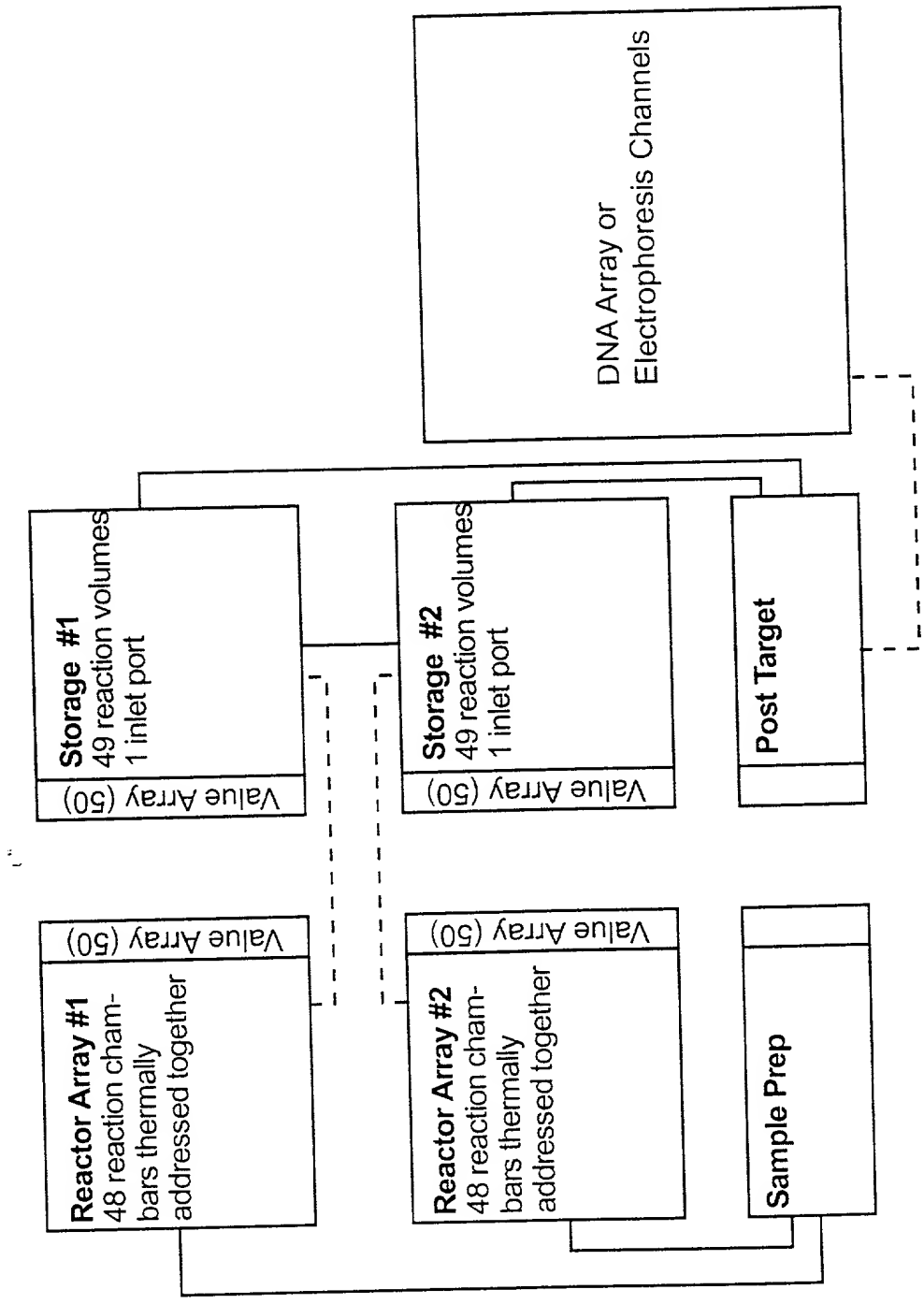

DNA Array or
Electrophoresis Channels
Storage #1
49 reaction volumes
1 inlet port
Value Array (50)
Storage #2
49 reaction volumes
1 inlet port
Value Array (50)
Post Target
Reactor Array #1
48 reaction cham-
bars thermally
addressed together
Value Array (50)
Reactor Array #2
48 reaction cham-
bars thermally
addressed together
Value Array (50)
Sample Prep

FIG. 13

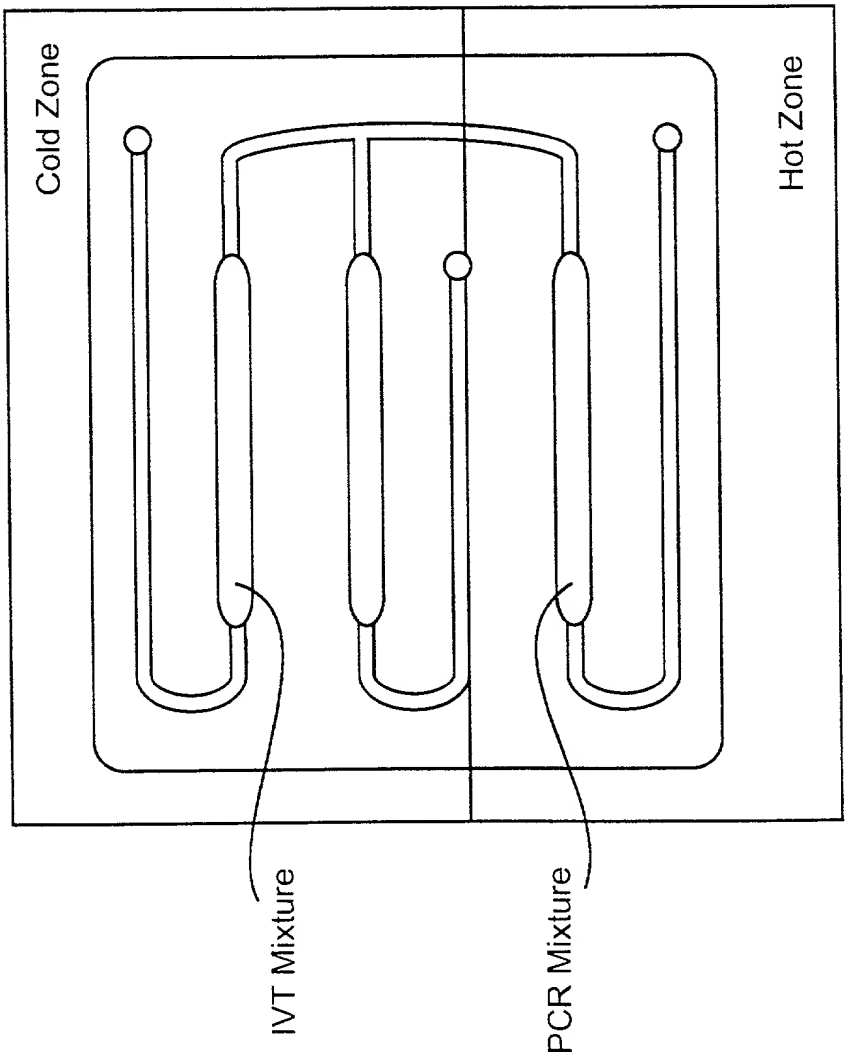


FIG. 14a

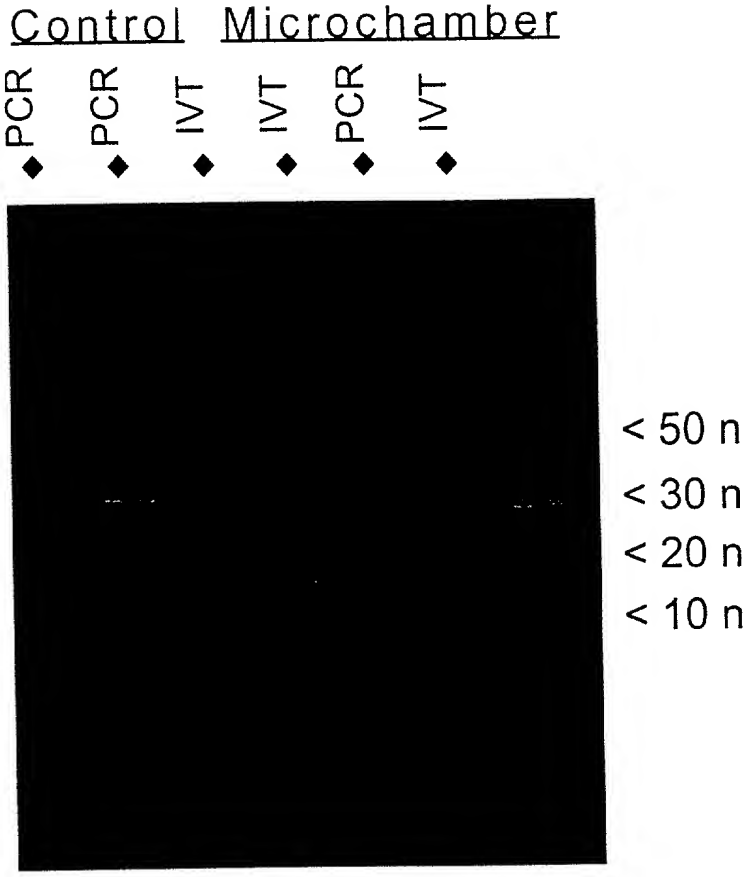


FIG. 14b

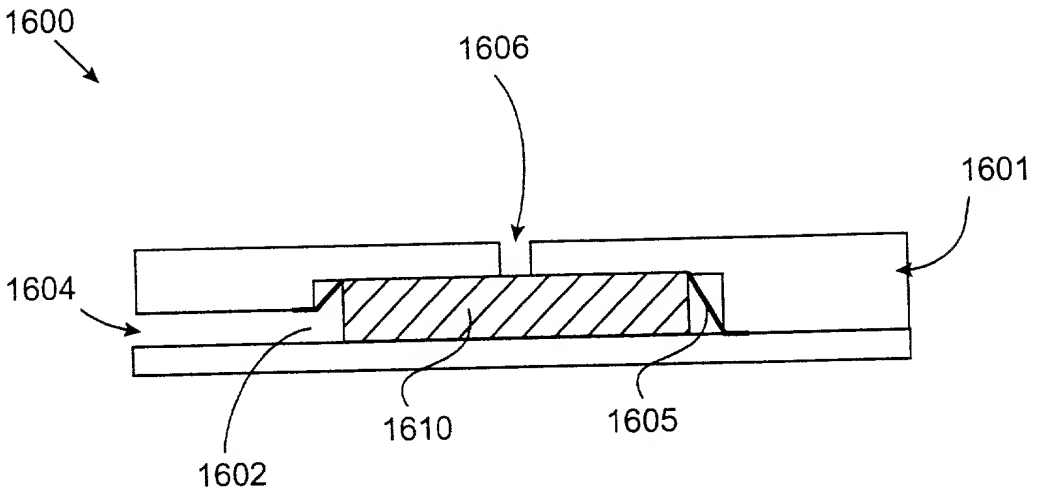

1600
1606
1601
1604
1610
1605
1602

FIG. 15

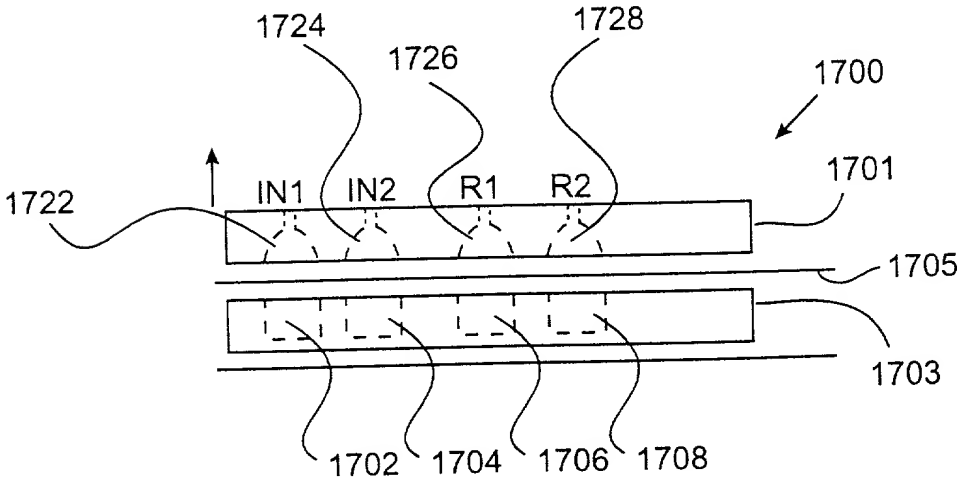

1724
1726
1728
1700
1722
IN1
IN2
R1
R2
1701
1705
1703
1702
1704
1706
1708

FIG. 16

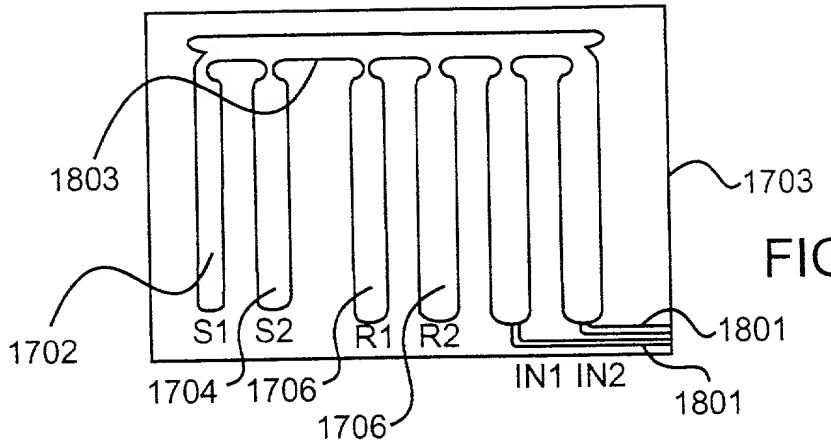


FIG. 17a

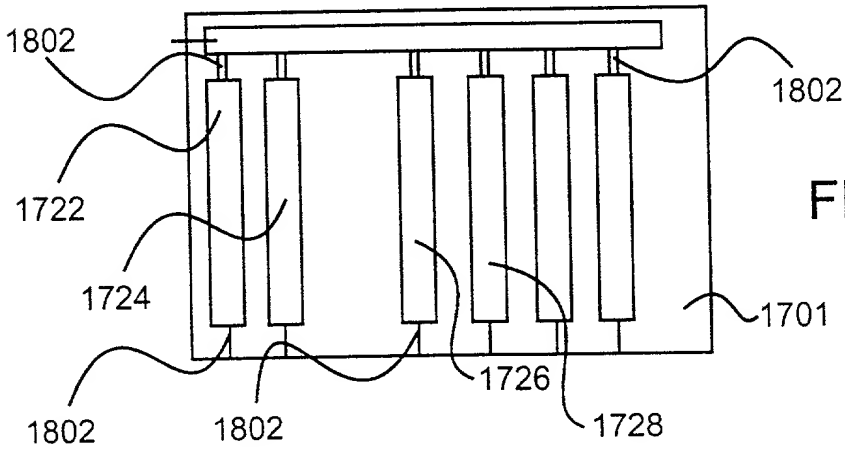


FIG. 17b

FIG. 17

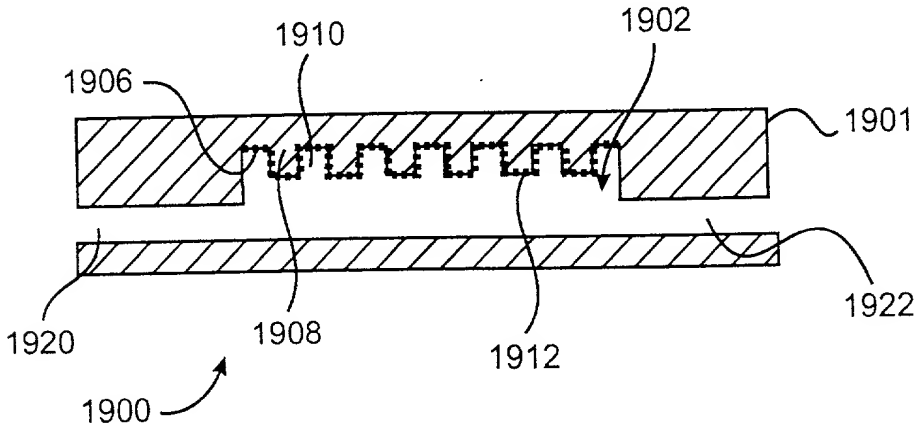


FIG. 18

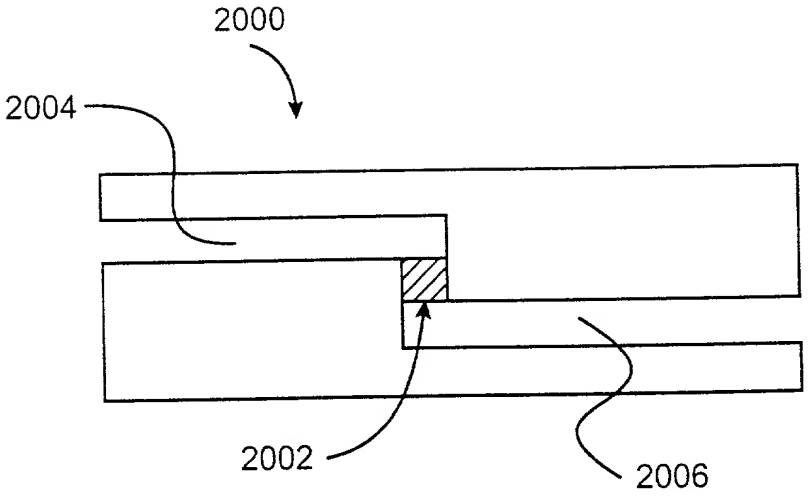


FIG. 19

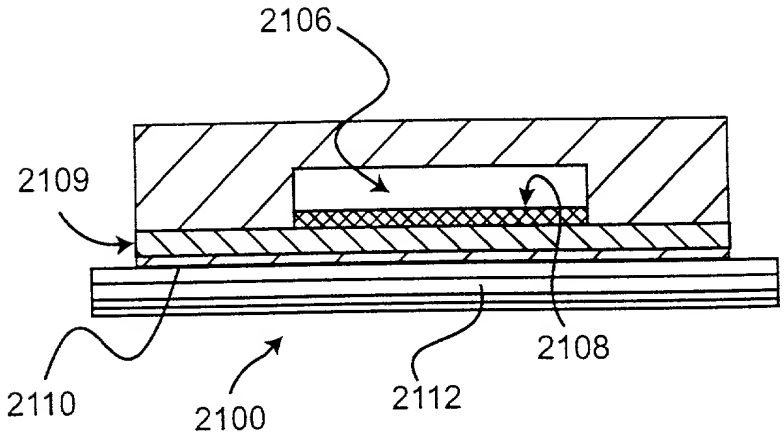


FIG. 20

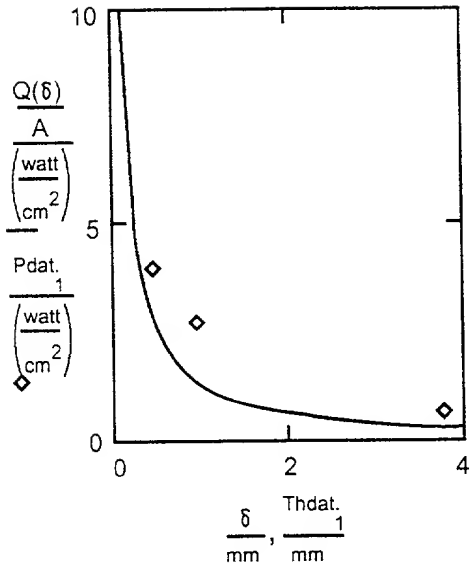


FIG. 21a

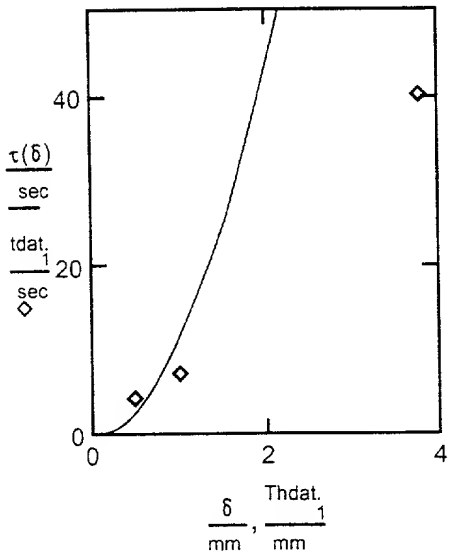


FIG. 21b

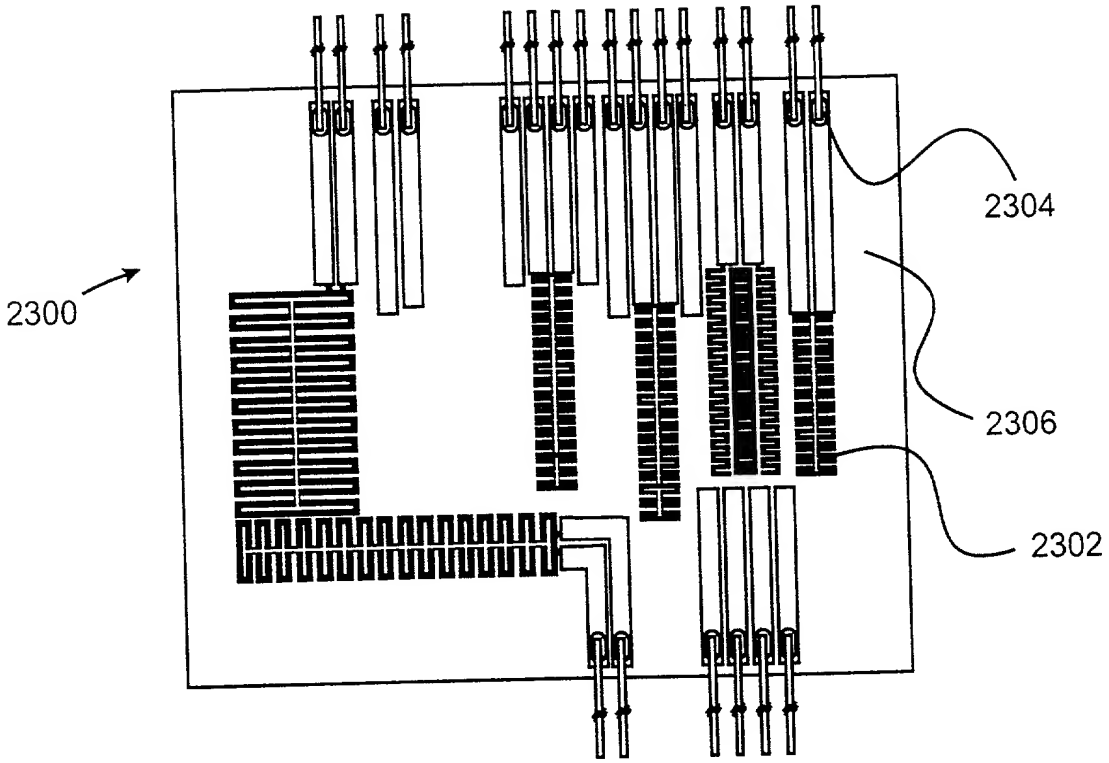

2300
2304
2306
2302

FIG. 22

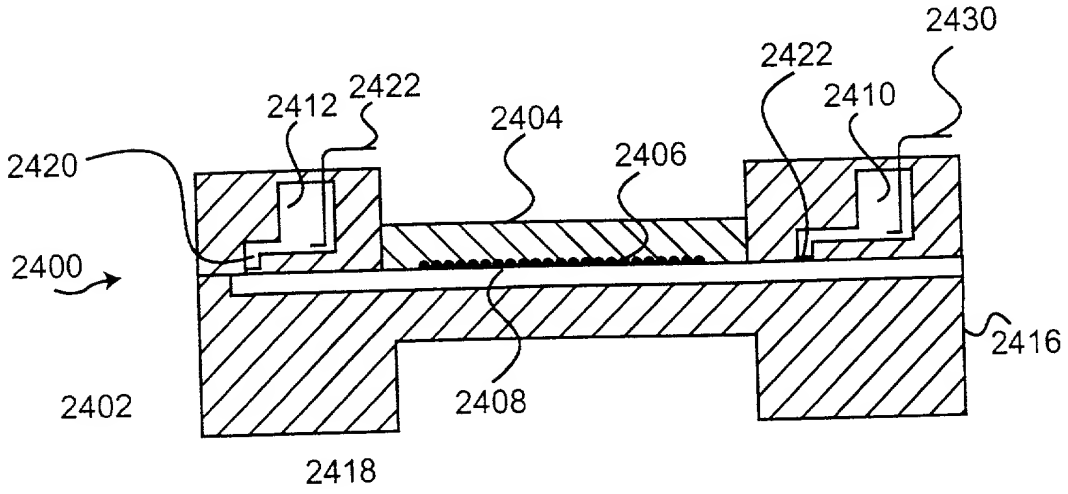

2430
2422
2412
2422
2410
2404
2420
2406
2400
2416
2402
2408
2418

FIG. 23

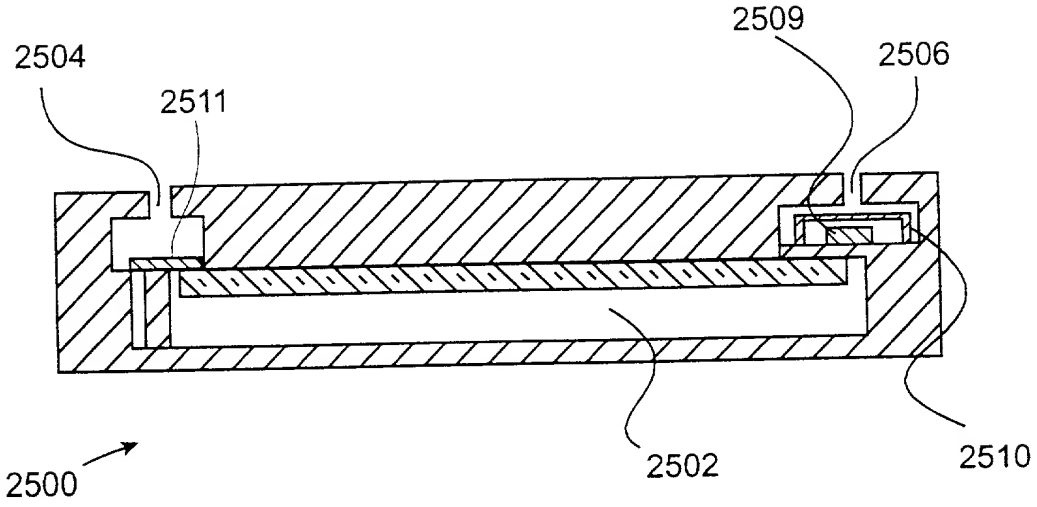


FIG. 24

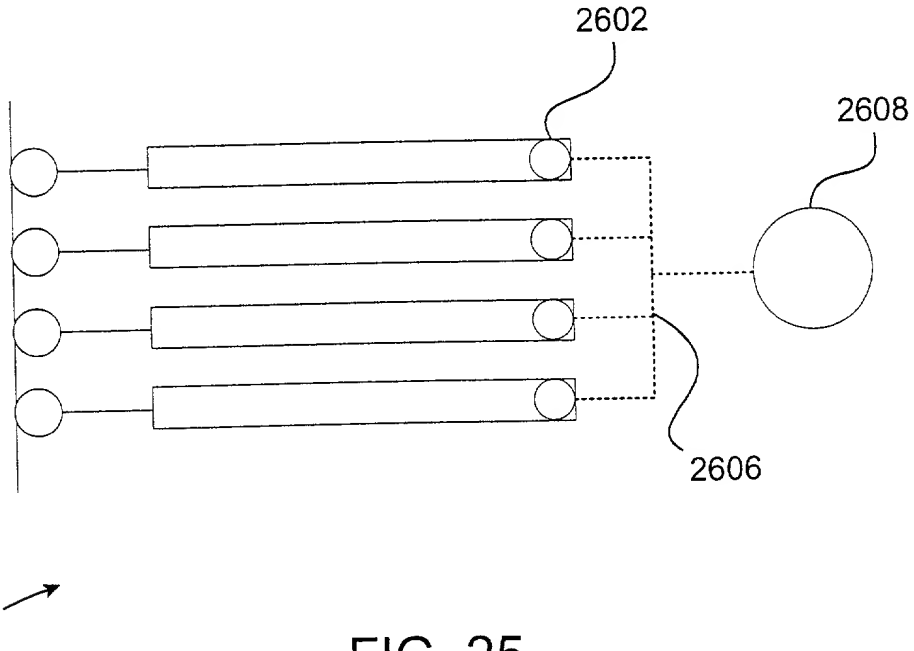


FIG. 25

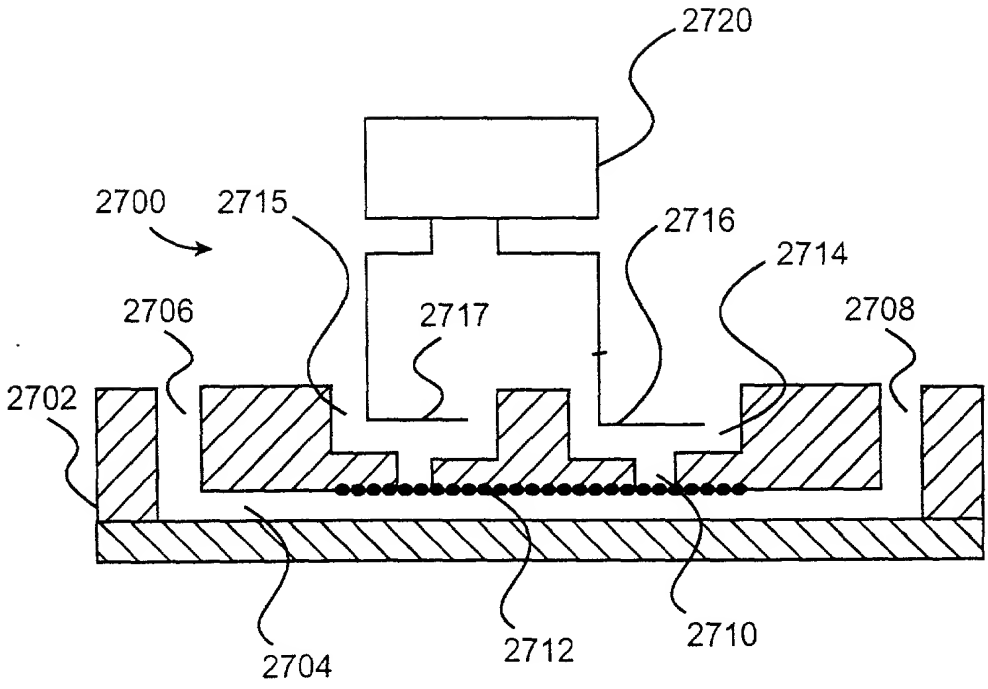


FIG. 26

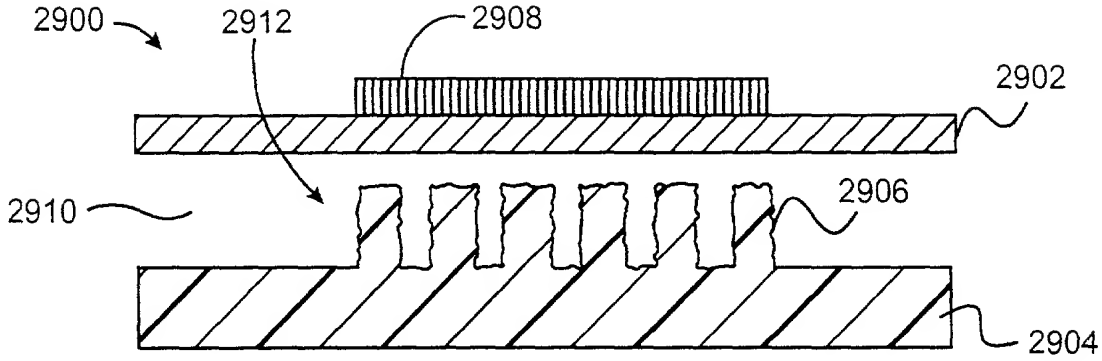

2900
2912
2908
2902
2910
2906
2904

FIG. 27

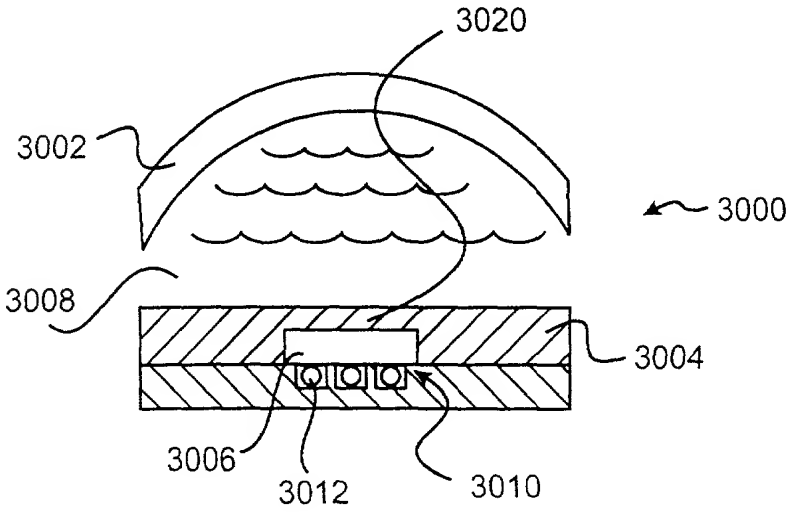

3020
3002
3000
3008
3004
3006
3012
3010

FIG. 28

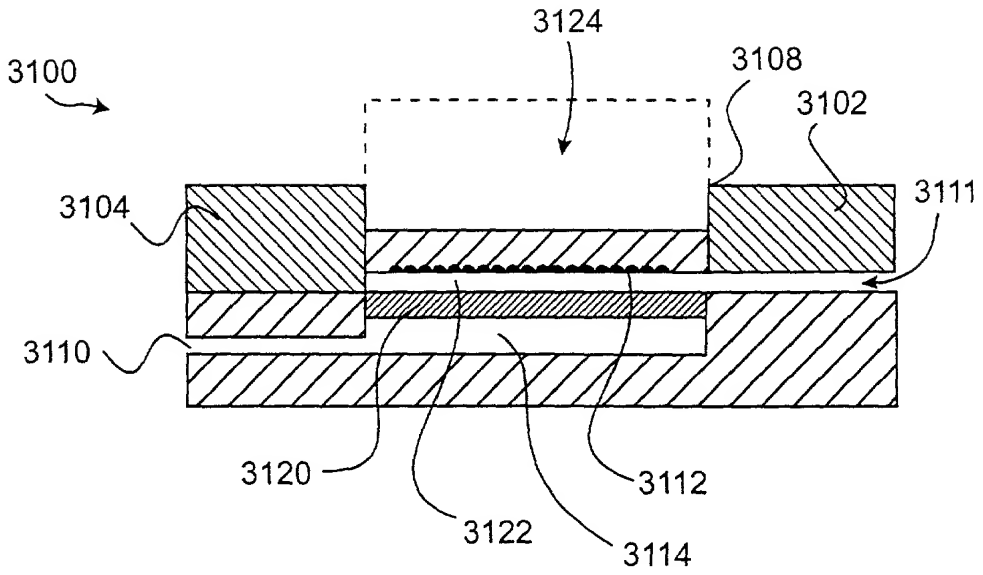


FIG. 29

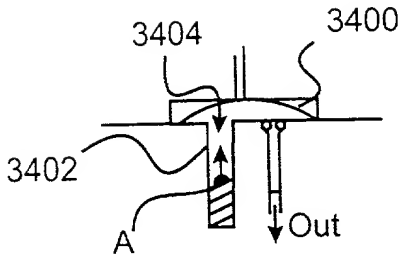


FIG. 30a

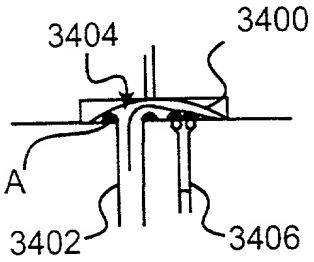


FIG. 30b

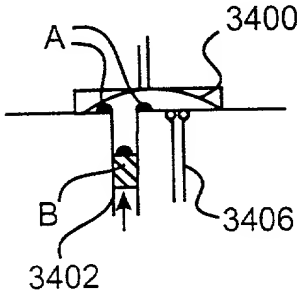


FIG. 30c

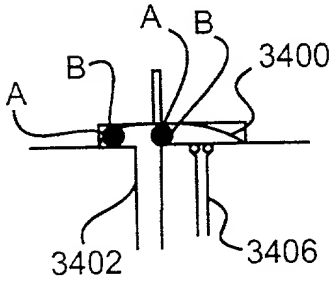


FIG. 30d

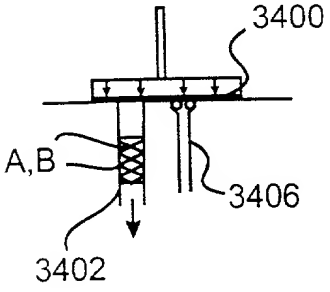


FIG. 30e

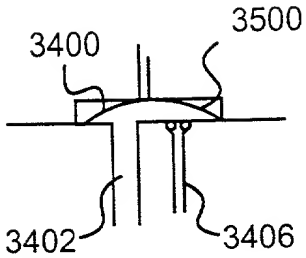


FIG. 31a

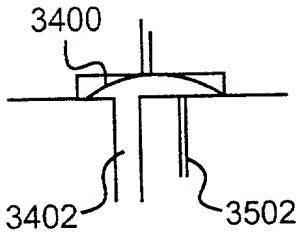


FIG. 31b

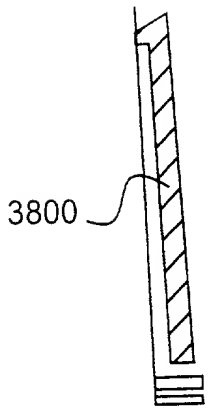


FIG. 32a

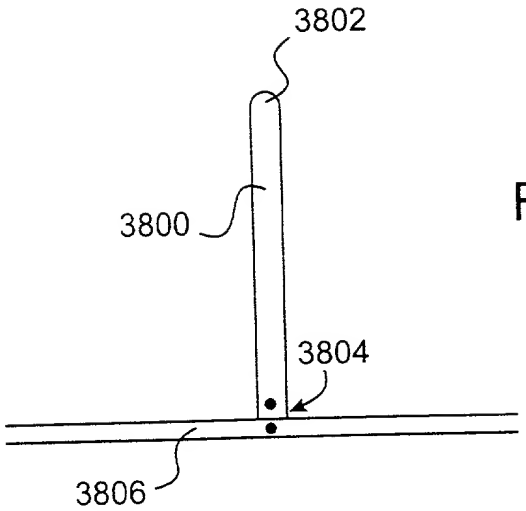


FIG. 32b

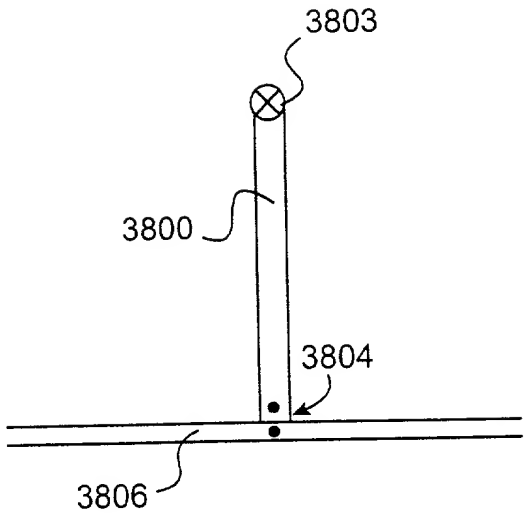


FIG. 32c

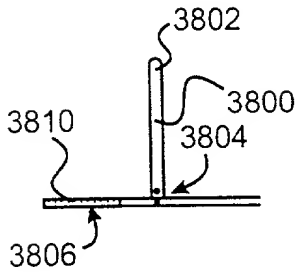


FIG. 33a

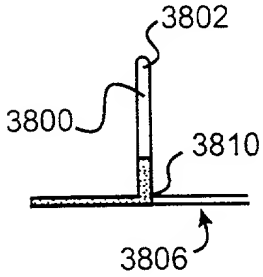


FIG. 33b

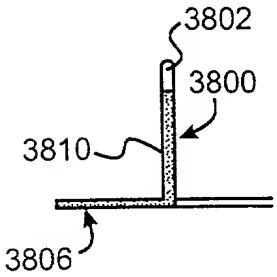


FIG. 33c

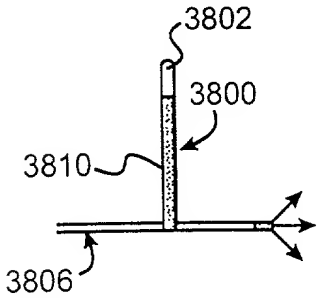


FIG. 33d

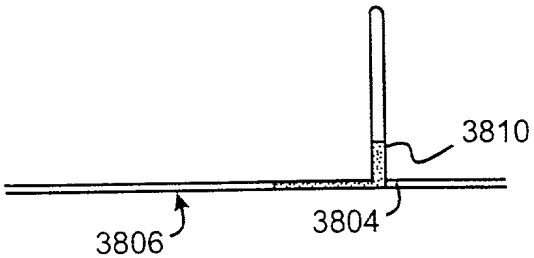


FIG. 33e

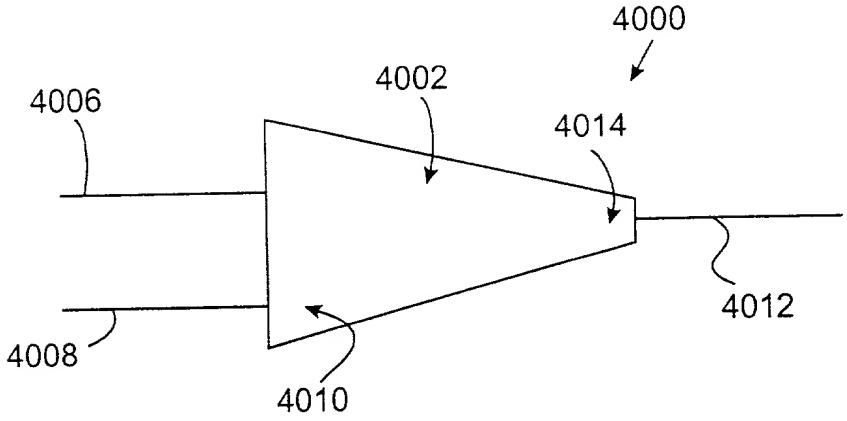


FIG. 34

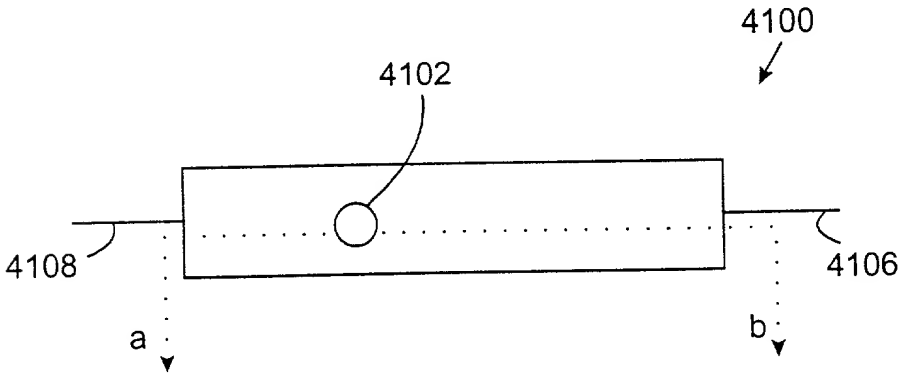


FIG. 35a

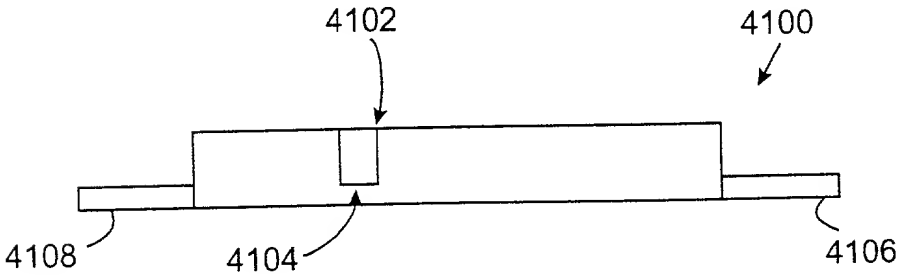


FIG. 35b

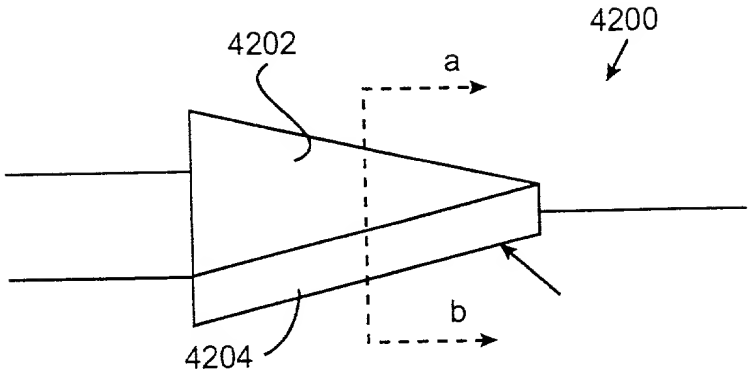


FIG. 36a

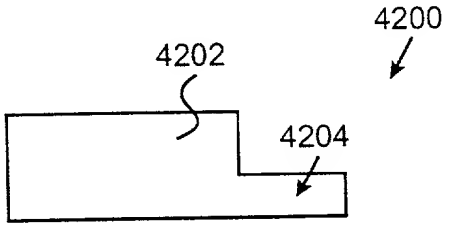


FIG. 36b

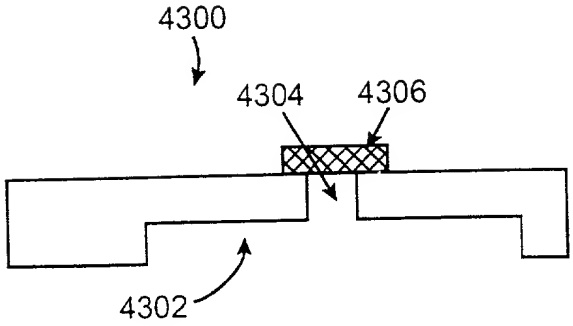


FIG. 37a

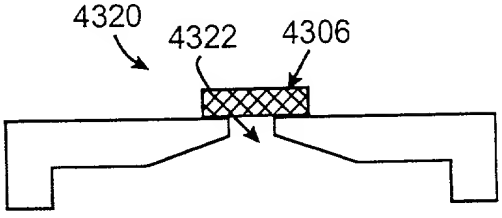


FIG. 37b

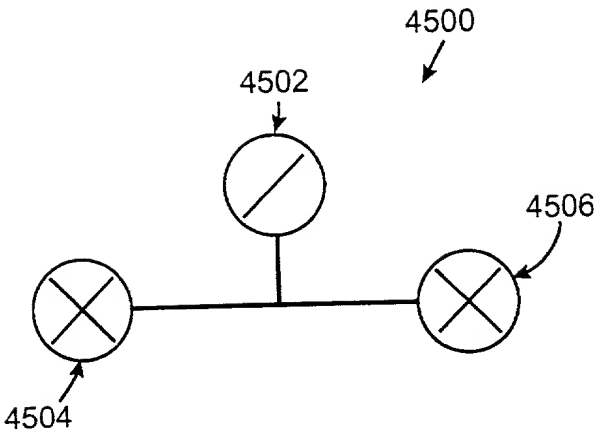


FIG. 38

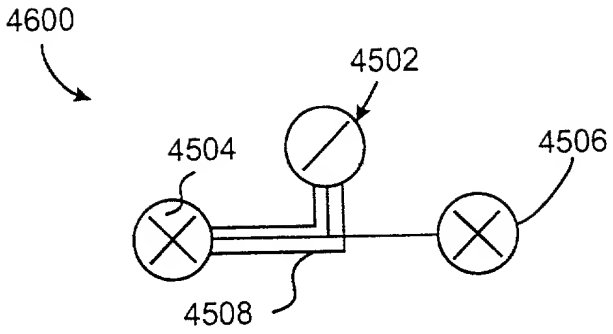


FIG. 39a

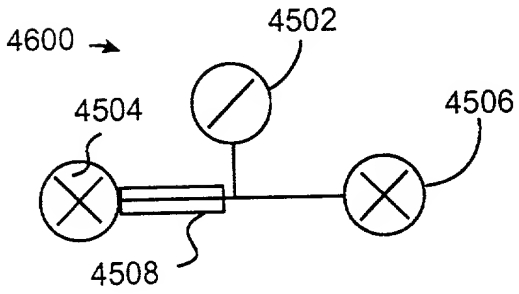


FIG. 39b

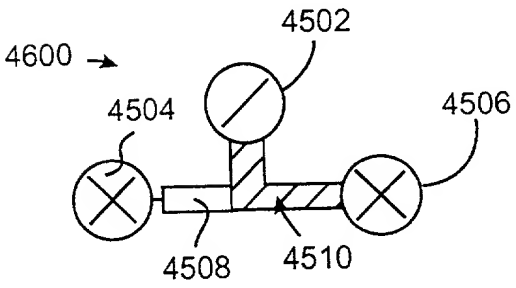
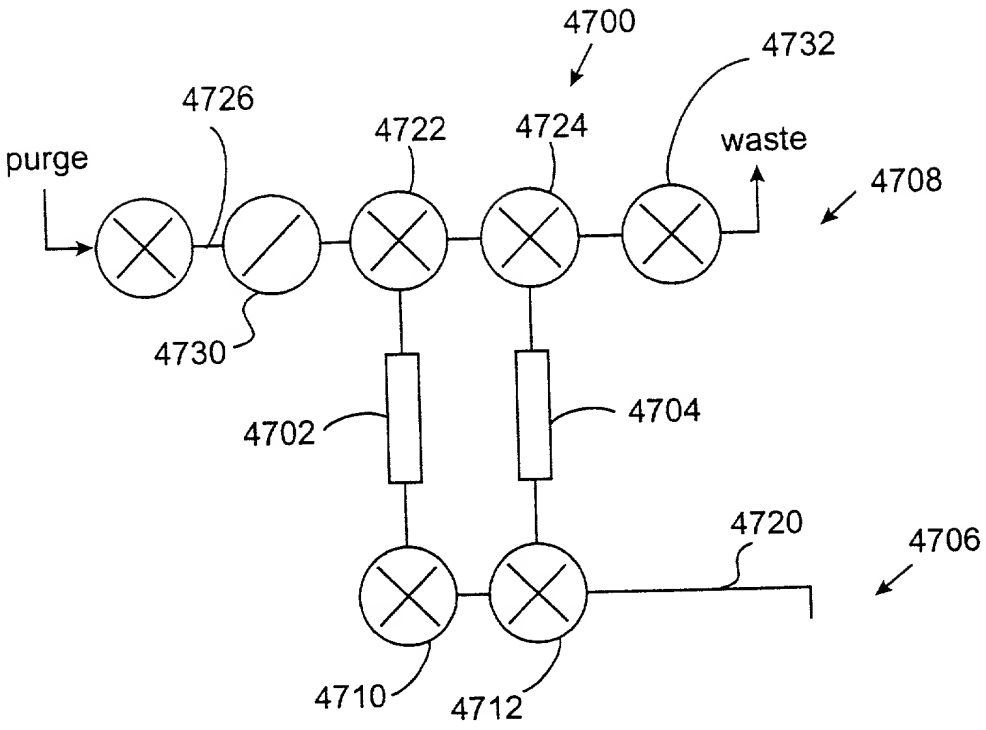


FIG. 39c


4700
4732
4726
4722
4724
waste
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4708
4730
4702
4704
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4706
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4712

FIG. 40

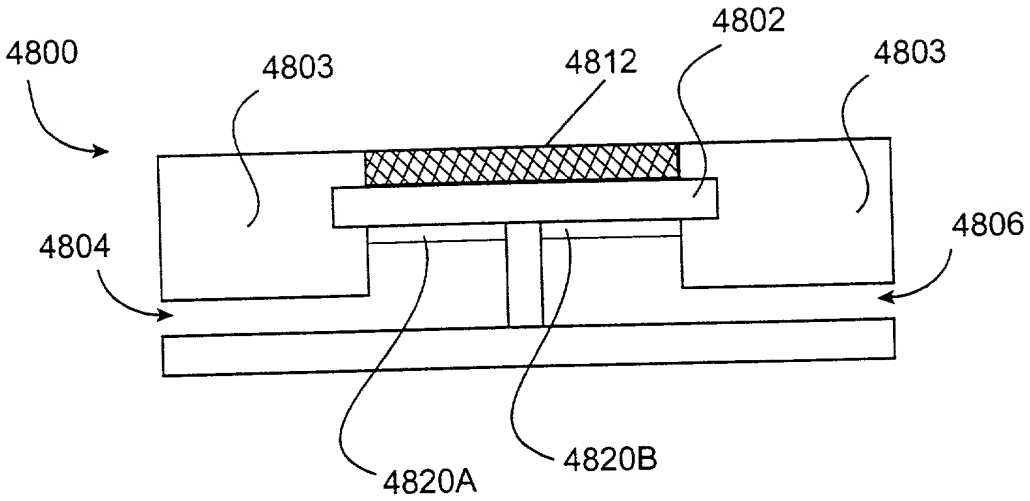


FIG. 41

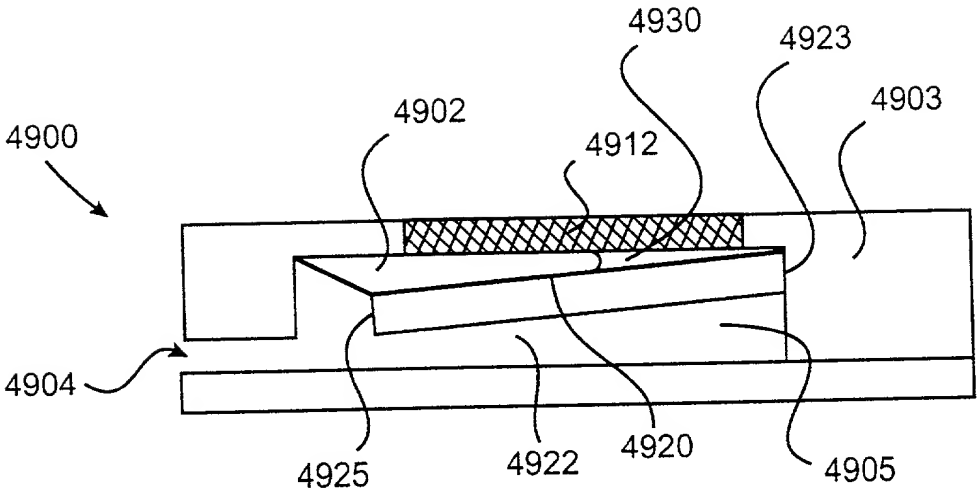


FIG. 42

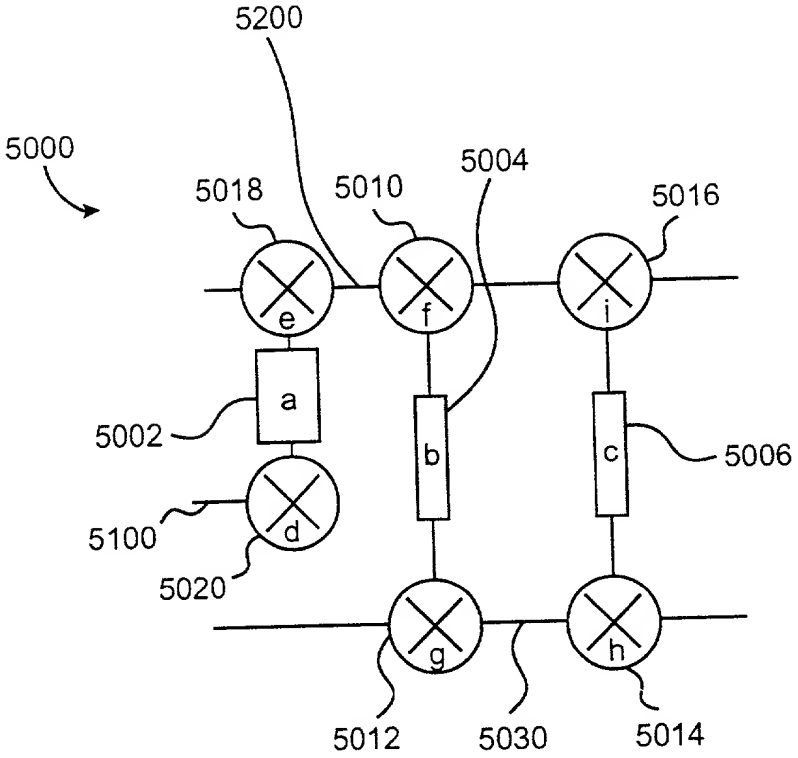

5200
5000
5018
5010
5004
5016
e
f
i
a
5002
b
c
5006
d
5100
5020
g
h
5012
5030
5014

FIG. 43

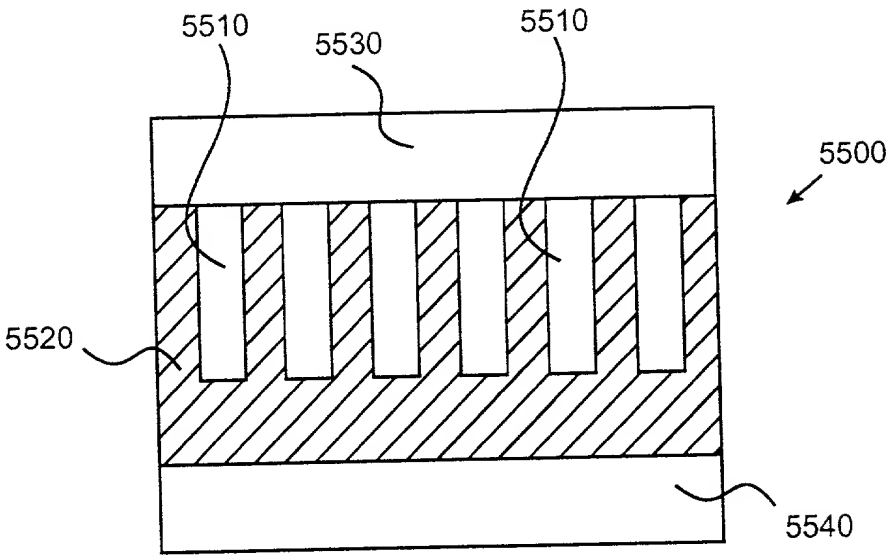


FIG. 44a

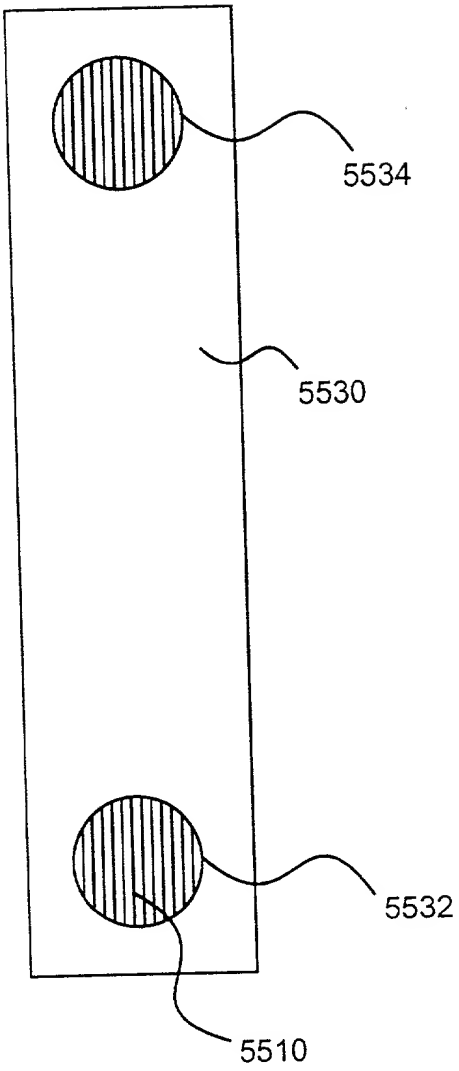


FIG. 44b

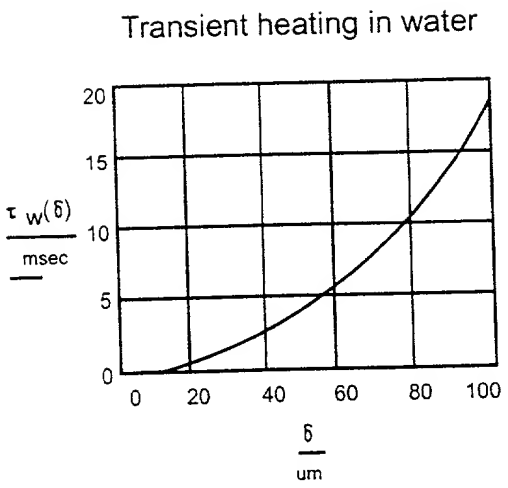


FIG. 44c

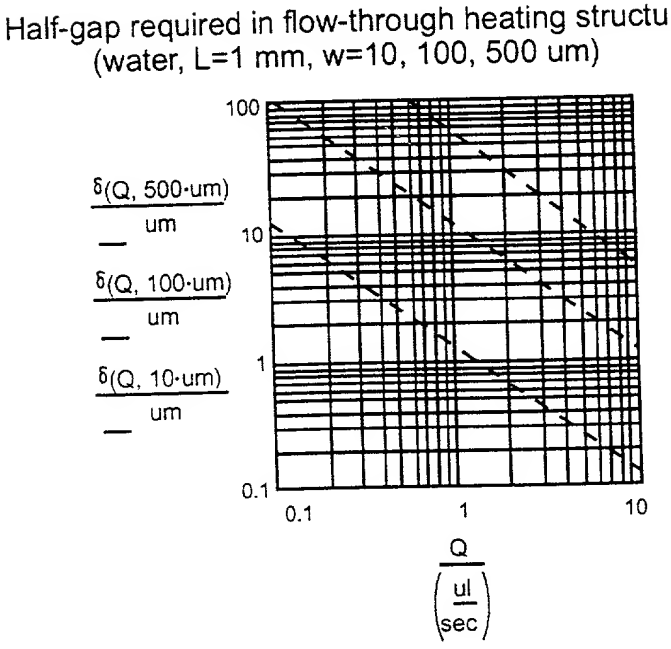


FIG. 44d

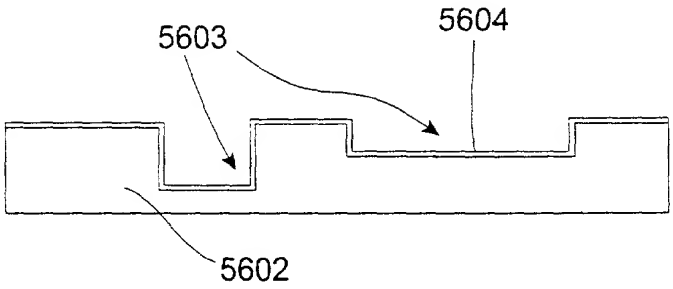


FIG. 45a

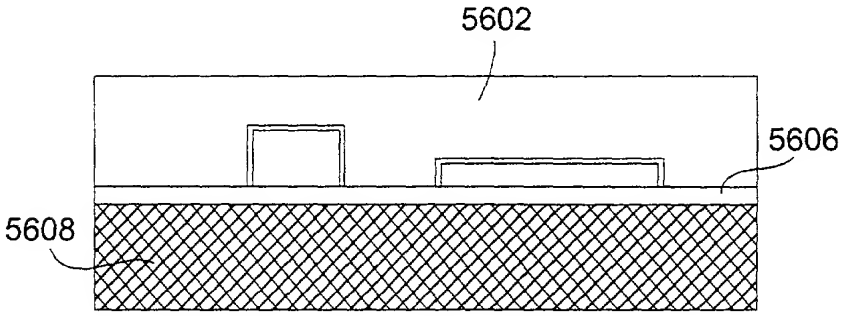


FIG. 45b

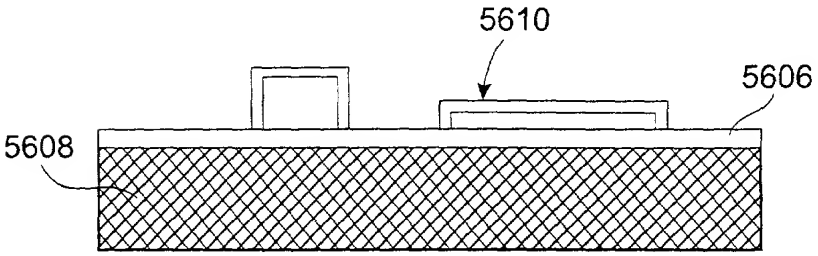


FIG. 45c

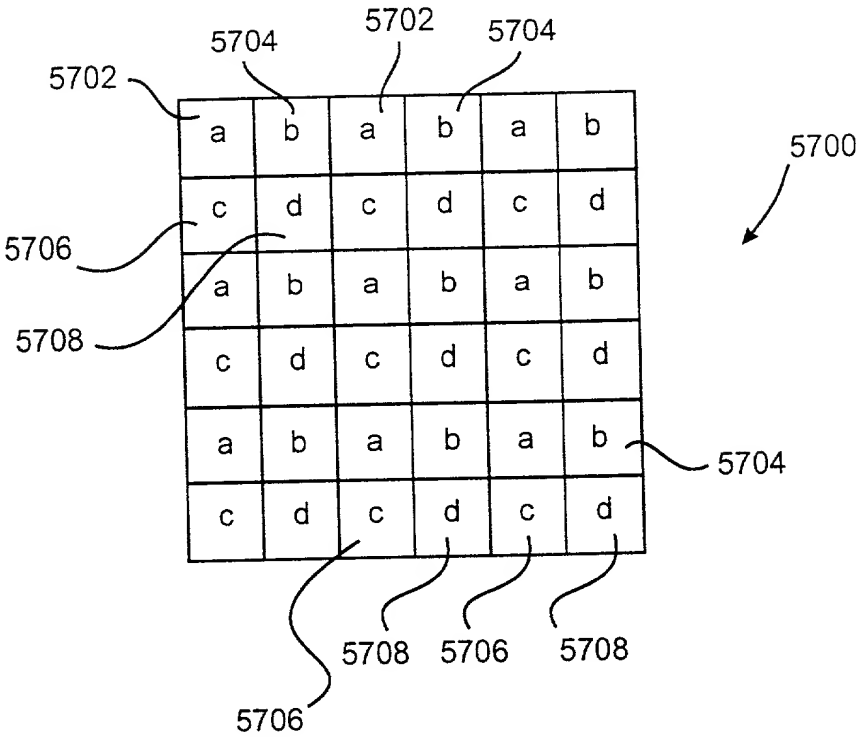


FIG. 46a

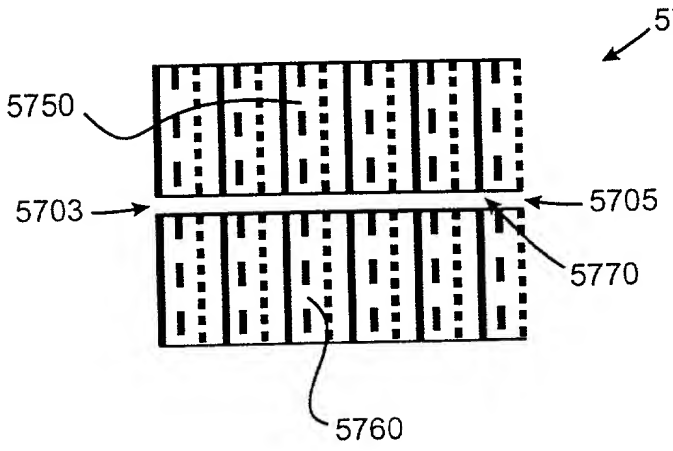


FIG. 46b

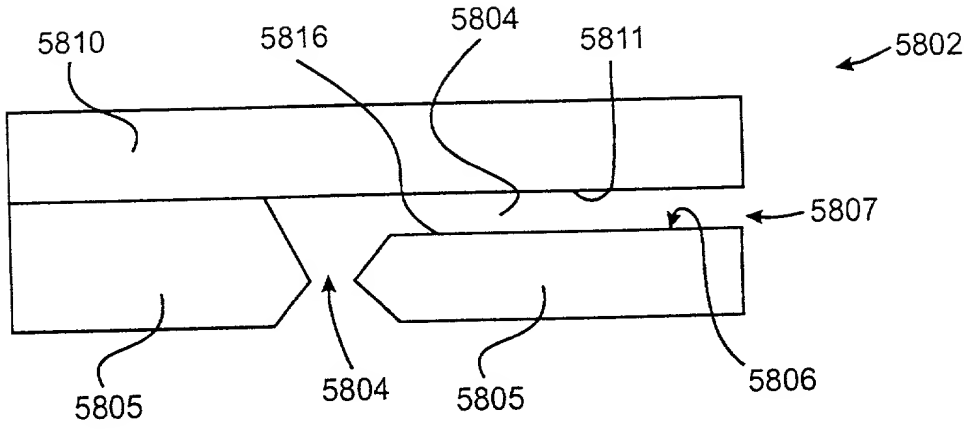


FIG. 47a

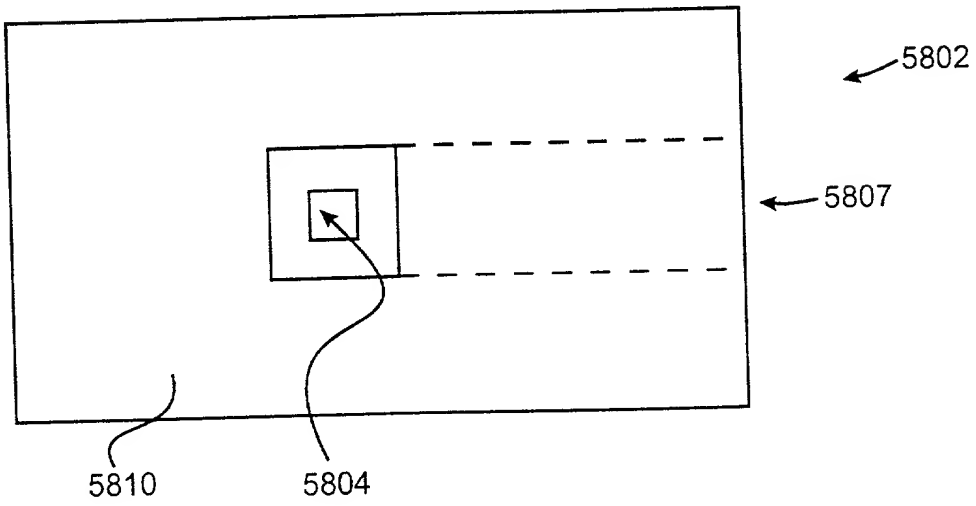


FIG. 47b

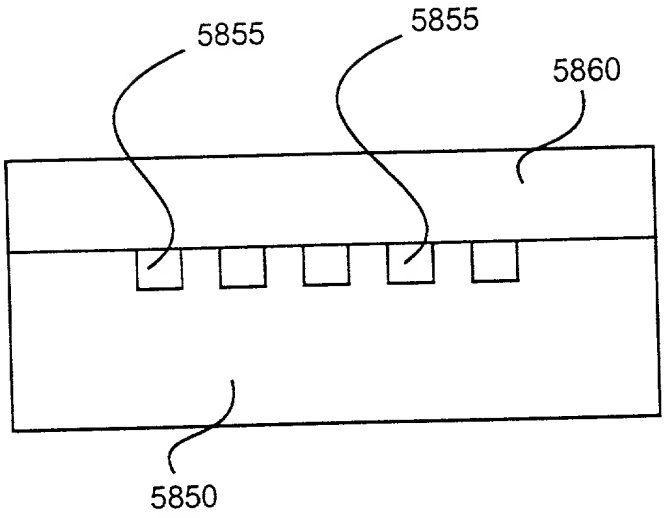


FIG. 47c

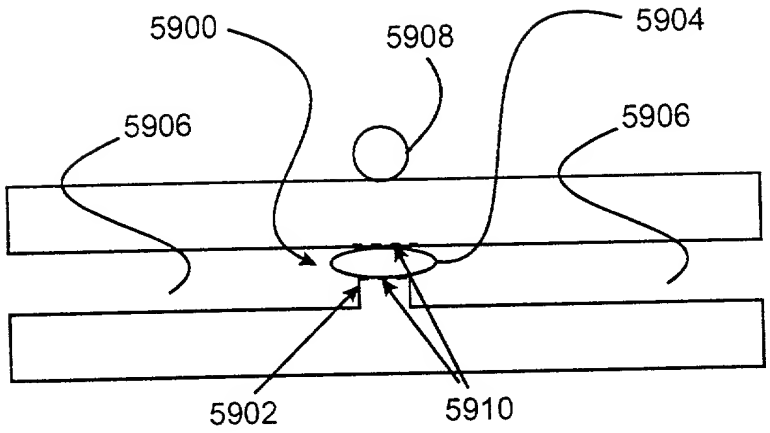


FIG. 48

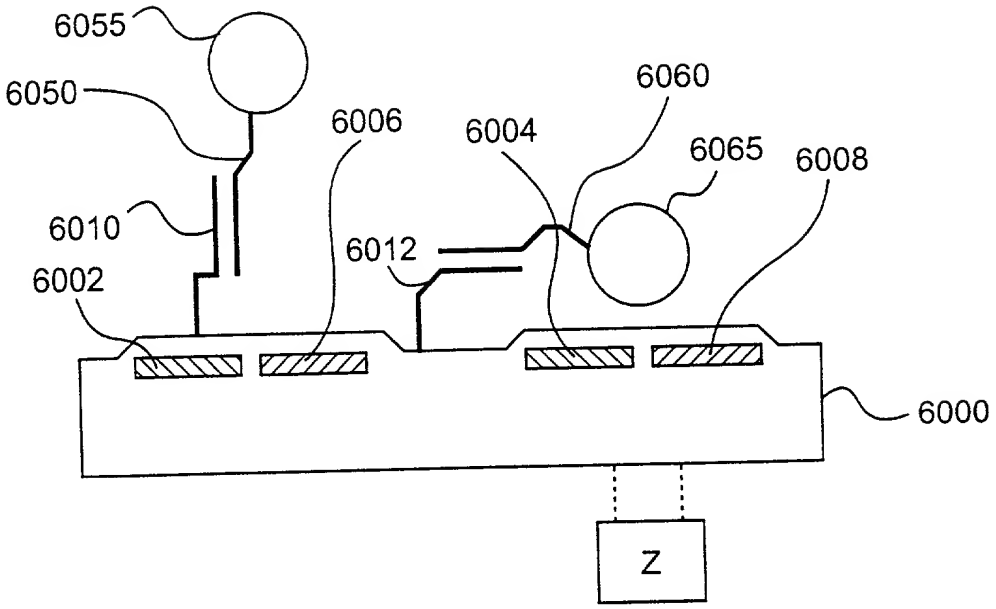
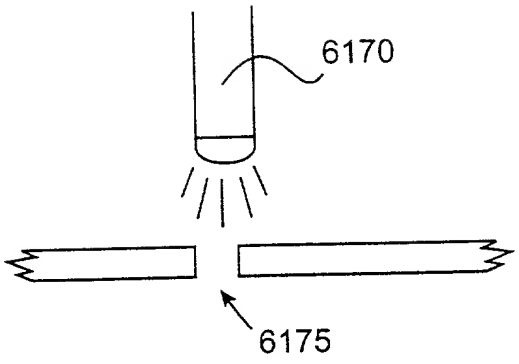


FIG. 49



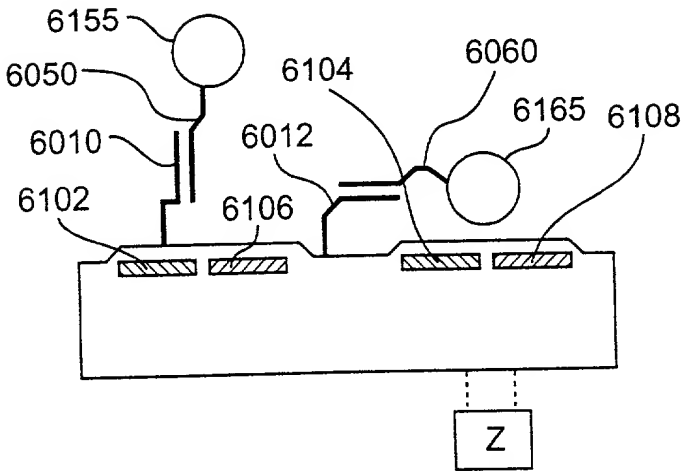


FIG. 50

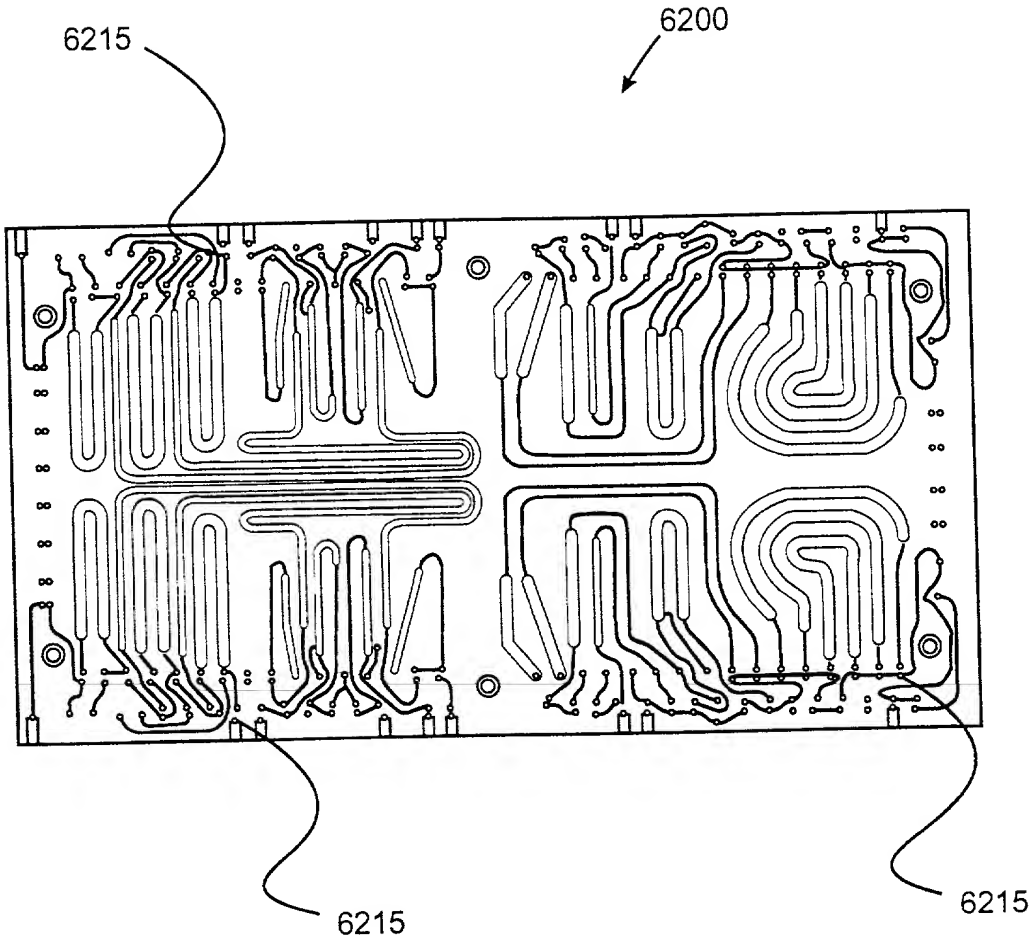


FIG. 51

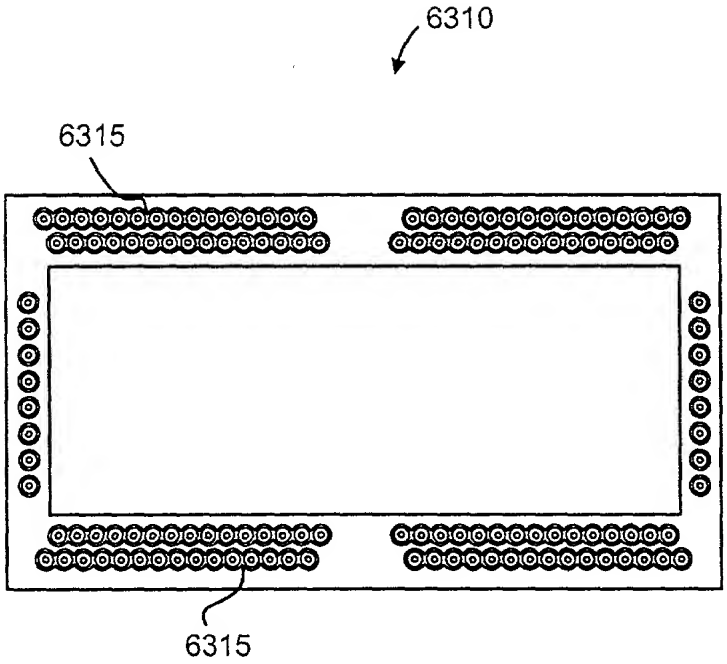


FIG. 52

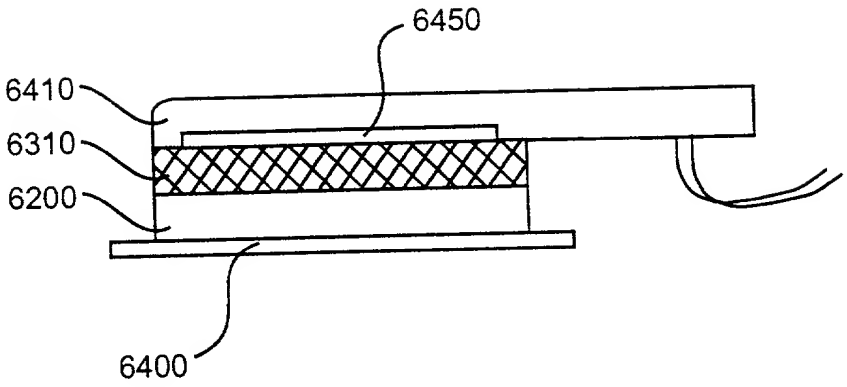


FIG. 53

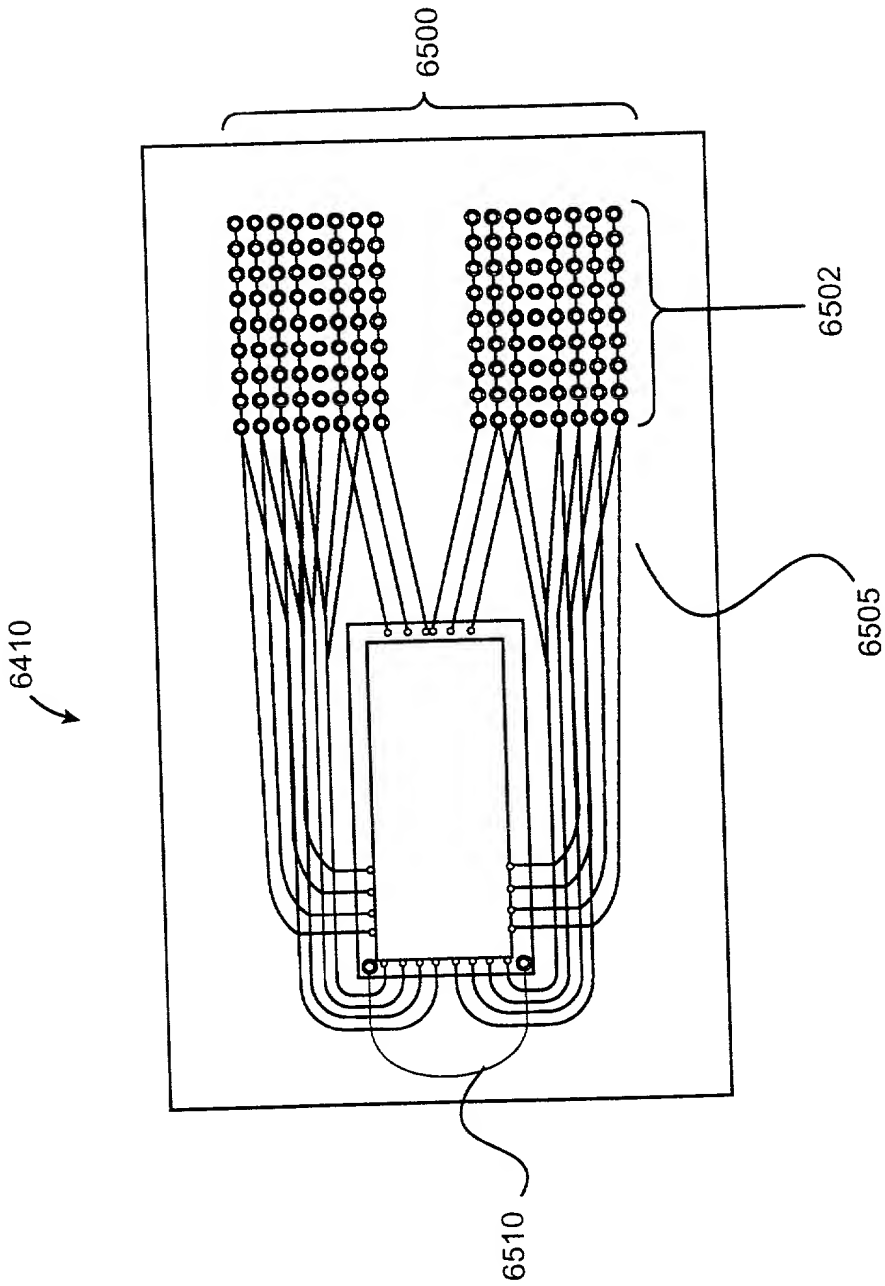


FIG. 54

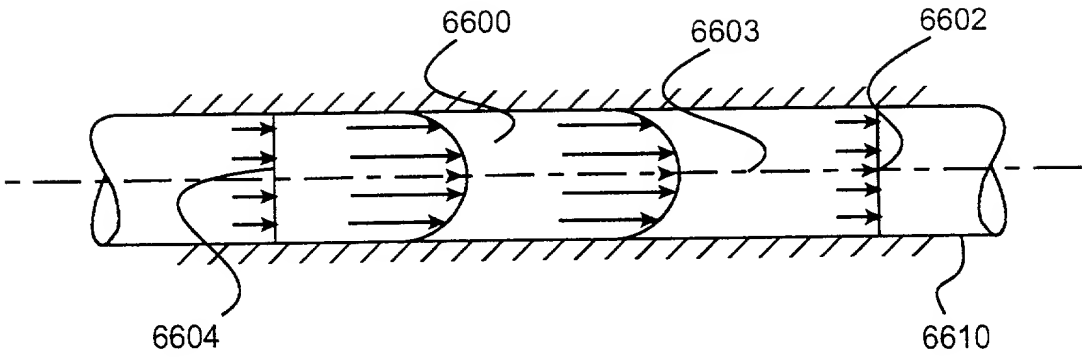


FIG. 55a

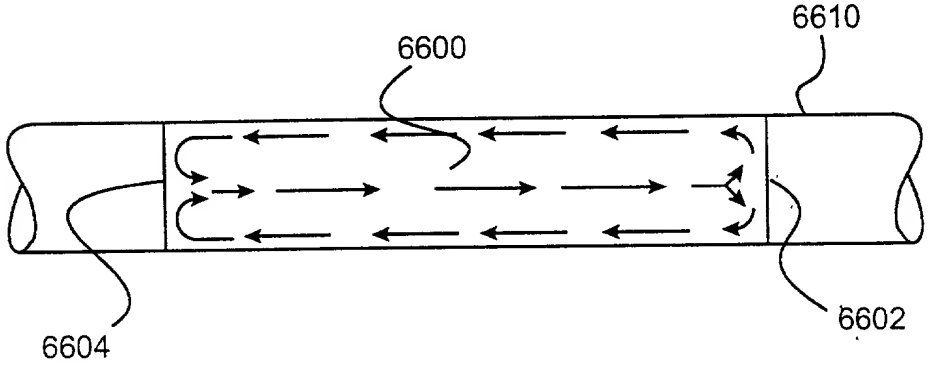


FIG. 55b

the genetic material of the cell. Further, the properties of these polypeptides, e.g., as enzymes, functional proteins, and structural proteins, are determined by the sequence of amino acids which make them up. As structure and function are integrally related, many biological functions may be explained by elucidating the underlying structural features which provide those functions, and these structures are determined by the underlying genetic information in the form of polynucleotide sequences. In addition to encoding polypeptides, polynucleotide sequences can also be specifically involved in, for example, the control and regulation of gene expression.

The study of this genetic information has proved to be of great value in providing a better understanding of life processes, as well as diagnosing and treating a large number of disorders. In particular, disorders which are caused by mutations, deletions or repeats in specific portions of the genome, may be readily diagnosed and/or treated using genetic techniques. Similarly, disorders caused by external agents may be diagnosed by detecting the presence of genetic material which is unique to the external agent, e.g., bacterial or viral DNA.

While current genetic methods are generally capable of identifying these genetic sequences, such methods generally rely on a multiplicity of distinct processes to elucidate the nucleic acid sequences, with each process introducing a potential for error into the overall process. These processes also draw from a large number of distinct disciplines, including chemistry, molecular biology, medicine and others. It would therefore be desirable to integrate the various process used in genetic diagnosis, in a single process, at a minimum cost, and with a maximum ease of operation.

Interest has been growing in the fabrication of microfluidic devices. Typically, advances in the semiconductor manufacturing arts have been translated to the fabrication of micromechanical structures, e.g., micropumps,

microvalves, and the like, and microfluidic devices including miniature chambers and flow passages.

A number of researchers have attempted to employ these microfabrication techniques in the miniaturization of some of the processes involved in genetic analysis in particular. For example, published PCT Application No. WO 94/05414, to Northrup and White, incorporated herein by reference in its entirety for all purposes, reports an integrated micro-PCR apparatus for collection and amplification of nucleic acids from a specimen. However, there remains a need for an apparatus which combines the various processing and analytical operations involved in nucleic acid analysis. The present invention meets these and other needs.

SUMMARY OF THE INVENTION

The present invention generally provides miniature integrated fluidic systems for carrying out a variety of preparative and analytical operations, as well as methods of operating these systems and methods of using these systems.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 shows a schematic representation of a nucleic acid diagnostic system for analysis of nucleic acids from samples.

Figs. 2A and 2B show schematic representations of two alternate reaction chamber designs from a cut-away view.

Fig. 3 shows a schematic representation of a miniature integrated diagnostic device having a number of reaction chambers arranged in a serial geometry.

Figs. 4A-C show a representation of a microcapillary electrophoresis device. Figs. 4A and 4B show the microcapillary configured for carrying out alternate loading strategies for the microcapillary whereas Fig. 4C illustrates the microcapillary in running mode.

Fig. 5A illustrates a top view of a miniature integrated device which employs a centralized geometry. Fig. 5B shows a side view of the same device wherein the central

chamber is a pumping chamber, and employing diaphragm valve structures for sealing reaction chambers.

Fig. 6 shows schematic illustrations of pneumatic control manifolds for transporting fluid within a miniature integrated device. Fig. 6A shows a manifold configuration suitable for application of negative pressure, or vacuum, whereas Fig. 6B shows a manifold configuration for application of positive pressures. Fig. 6C illustrates a pressure profile for moving fluids among several reaction chambers.

Fig. 7A shows a schematic illustration of a reaction chamber incorporating a PZT element for use in mixing the contents of the reaction chamber. Fig. 7B shows mixing within a reaction chamber applying the PZT mixing element as shown in Fig. 7A. Fig. 7C is a bar graph showing a comparison of hybridization intensities using mechanical mixing, acoustic mixing, stagnant hybridization and optimized acoustic mixing.

Fig. 8 is a schematic illustration of a side and top view of a base-unit for use with a miniature integrated device.

Fig. 10A is a gel showing a time course of an RNA fragmentation reaction. Fig. 10B is a gel showing a comparison of the product of an in vitro transcription reaction in a microchamber vs. a control (test tube). Fig. 10C is a comparison of the PCR product produced in a PCR thermal cycler and that produced by a microreactor.

Fig. 11 shows an embodiment of a reaction chamber employing an electronic pH control system.

Figs. 12A-C show a schematic representation of a miniature integrated device employing a pneumatic fluid direction system utilizing a gas permeable fluid barrier bound vents, e.g., a poorly wetting or hydrophobic membrane, and pneumatically controlled valves. Fig. 12A shows an embodiment of a single chamber employing this system. Fig. 12B is a schematic illustration of a debubbling chamber for linking discrete fluid plugs that are separated by a gas bubble. Fig. 12C schematically illustrates this system in an integrated device having numerous chambers, including degassing chamber,

dosing or volumetric chamber, storage and reaction chambers. Fig. 12D is an illustration of an injection molded substrate which embodies the system schematically illustrated in Fig. 12C.

Fig. 13 is a schematic representation of a device configuration for carrying generic sample preparation reactions.

Fig. 14 is a schematic representation of a device configuration for carrying out multiple parallel reactions.

Fig. 15 shows a demonstration of integrated reactions in a microfabricated polycarbonate device. Fig. 15A shows the layout of the device including the thermal configuration of the device. Fig. 15B shows the results of PCR amplification and subsequent in vitro transcription within the chambers of the device.

Fig. 16 schematically illustrates a deformable high capacity nucleic acid extraction device incorporating a porous material for extracting nucleic acids from samples.

Fig. 17 is a side sectional view of a miniaturized reactor device incorporating a positive displacement fluid movement scheme.

Fig. 18A is a top plan view of the pneumatic portion of the reactor device of Fig. 17.

Fig. 18B is a top plan view of the fluid portion of the reactor device of Fig. 17.

Fig. 19 schematically illustrates an affinity based nucleic acid extraction device incorporating a textured wall.

Fig. 20 illustrates an allele-specific purification device according to the present invention.

Fig. 21 is a schematic representation of a miniaturized device for performing rapid thermal cycling reactions, such as PCR or RT-PCR.

Figs. 22A and 22B are graphs of steady state power and cooling time versus thermal insulator thickness, respectively, for the device of Fig. 21.

Fig. 23 is a top view of an array of thin-film heaters mounted on a single thermoelectric cooler for independent

rapid thermal cycling reactions in the miniature device of Fig. 21.

Fig. 24 is a cross-section view of a hybridization cartridge.

Fig. 25 is a schematic illustration of a sealed pneumatic cartridge having a deformable diaphragm for drawing fluid into or ejecting fluid from a chamber.

Fig. 26 schematically illustrates an array of sealed pneumatic chambers on disposable cartridges.

Fig. 27 is a cross-sectional view of an electrically controlled nucleic acid purification chamber.

Fig. 29 is a cross-sectional view of a miniaturized m-RNA purification system.

Fig. 30 is a sectional view of a cell lysis or nucleic acid fragmentization system incorporating acoustic energy.

Fig. 31 is a partial sectional view of a cartridge adapted for low volume hybridization of high density oligonucleotide arrays.

Figs. 34A-34E illustrate a system and method for linking two fluid plugs.

Figs. 35A and 35B illustrate alternative embodiments of the system of Figs. 34A-34E.

Figs. 38A, 38B and 38C illustrate a chamber adapted for measuring or metering a variable amount of fluid.

Figs. 39A-39E illustrate a method for measuring a fluid amount with the chamber of Figs. 38A and 38B.

Fig. 40 illustrates a tapered chamber for linking fluid plugs with surface tension.

Figs. 41A and 41B illustrate a stalactite chamber for linking fluid plugs with surface tension.

Figs. 42A and 42B illustrate a chamber having a shallow region for linking fluid plugs with surface tension.

Fig. 43A illustrates a previous fluid mixing/linking structure with a vent membrane.

Fig. 43B illustrate the inventive fluid mixing/linking structure with a tapered channel leading to the vent membrane.

Figs. 44A and 44B **REMOVED**